Giovanni Corso
Paolo Veronesi
Franco Roviello *Editors*

Hereditary Gastric and Breast Cancer Syndrome

CDH1: One Genotype with Multiple Phenotypes

Springer

Hereditary Gastric and Breast Cancer Syndrome

Giovanni Corso • Paolo Veronesi •
Franco Roviello
Editors

Hereditary Gastric and Breast Cancer Syndrome

CDH1: One Genotype with Multiple Phenotypes

Springer

Editors
Giovanni Corso
European Institute of Oncology
University of Milan
Milan, Italy

Paolo Veronesi
European Institute of Oncology
University of Milan
Milan, Italy

Franco Roviello
University of Siena
Siena, Italy

ISBN 978-3-031-21316-8 ISBN 978-3-031-21317-5 (eBook)
https://doi.org/10.1007/978-3-031-21317-5

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

A beautiful life dedicated to cancer research.
In memory of ***Raquel Seruca*** *(1962–2022)*

Foreword

Any oncologist would agree that the dissimilarity between cancer of the stomach and breast excels all others. Etiology, prognosis, pathophysiology, and treatment, for example, differ in such a way that one can rightfully speak of two entirely different diseases. Besides, they originate in organs far away from each other in the human body with no direct or indirect relationship. The purpose, control, and functions are quite different as well. On top, breasts start to grow at the onset of puberty, where the stomach functions already in utero. Indeed, both are at the ends of the spectrum.

Non-cardiac gastric cancer can be subdivided into two distinct pathologic entities, intestinal and diffuse, which have different epidemiologic and prognostic features. The latter leads to gastric oncogenesis through the E-cadherin–catenin complex, which plays a critical role in the maintenance of normal tissue architecture. Mutation of any of its components results in the loss of cell-to-cell adhesion, thereby contributing to neoplasia. E-cadherin/*CDH1* gene germline mutations have been recognized in families with an inherited predisposition to diffuse gastric cancer. Amplification and/or overexpression of putative trophic factors have also been observed in gastric cancer. Finally, *Helicobacter pylori* (*H. pylori*) infection is also involved through various mechanisms. Gastric cancer is traditionally linked to poor food preservation and food infections. Apart from refrigeration and freezing, food can be preserved through smoke, salt, and against clostridium with nitrates. All these additives prove carcinogenic in one way or the other. In those regions in the world where refrigeration is possible, gastric cancer declines. Electricity and hygiene are in this sense essential to prevent cancer. Avoidance of helicobacter infection or eradication is another way. Treatment of local cancer is surgery with, in some well-defined cases, radiotherapy. Systemic adjuvant treatment conveys little benefit. In case of metastatic disease, only chemotherapy or, in some cases, targeted therapy can be offered. But prognosis remains universally poor with only 30% of patients surviving the first 5 years.

The epidemiology of breast cancer is entirely different and mainly related to pubertal developmental interferences on the target organ. Ionizing irradiation, calorie-rich nutrition, (xeno-) estrogens, and poor physical activity are all involved and have major influence on the developing organ. Screening and early detection are pivotal and, once occurred, local surgery with or without radiotherapy is the cornerstone of a most effective treatment. The benefit of adjuvant treatments is well established. And for systemic disease a plentitude of treatments is available,

depending on the molecular profile. Prognosis is quite good in most patients with a disease-free survival amounting to beyond 80% after 5 years.

And yet, this book deals about similarities.

These two extremes in oncology share an interesting origin that becomes evident during oncogenesis. Both gastric and breast cancer are prevalent and mortalities in absolute numbers score high without much variation in the last decade. Both cancers are a collection of different diseases and will generate alternative signal regulating pathways as many other cancers might do. But the similarity is deeper, at the genomic level. Both share a unique germline mutation that puzzled researchers for already several decades now.

More than any work before, the content of this work illustrates clearly how modern oncologists look at clinically different diseases. Central is the mutation in the *CDH1* gene that causes an autosomal-dominant gastric and breast cancer syndrome. The mutational variants can be classified into the usual missense, non-sense, splicing, insertions, and deletions. In diffuse gastric tumors, the predominant defects are exon skipping, which causes in-frame deletions. By contrast, most mutations found in infiltrating lobular breast cancers are out-of-frame mutations, which are predicted to yield secreted truncated E-cadherin fragments.

CDH1 encodes for E-cadherin, which is an essential molecule for epithelial homeostasis and control of cell adhesion. Individuals with germline mutations in the *CDH1* gene consistently demonstrated absence of loss of heterozygosity, suggesting the hypothesis that *CDH1* promoter methylation might function as the "second genetic hit" in carcinogenesis. Loss or aberrant expression results in disturbed cell-to-cell adhesion, increased cell invasion, and metastasis. Myosin V and F-actin, and many other factors, are required for the formation of a continuous apicolateral E-cadherin layer, the zonula adherens. When this is missing because of mutation in the *CDH1* gene, subsequent diseases can develop.

Compelling experimental evidence exists for a potent invasion suppressor role of this cell-to-cell adhesion molecule E-cadherin. In addition, a tumor suppressor effect has been suggested. Partial or complete loss of E-cadherin expression correlates with malignancy. Loss of E-cadherin expression increases diffuse growth pattern in both lobular and ductal types of breast cancer and to some extent in other cancers. Indeed, *CDH1* are also found in a small, clinically unimportant, proportion of colorectal cancers. The diffuse growth pattern is well illustrated in typical histological features such as the "Indian file" growth pattern.

One of the main questions is: Why mostly the breast and stomach are affected while germline mutation confers a risk in all organs? For sure, the relationship between the mutation and cancer is too simplistic to explain oncogenesis in both organs and a "first hit" has to be considered. In the breast, one can think of the hormonal environment with epigenetic imprinting, which is known to be carcinogenic. In the stomach, *CDH1* promoter methylation is the second hit in more than half of the sporadic diffuse gastric carcinoma cases harboring *CDH1* mutations. This "second hit" theory is well received when considering that initiation precedes promotion in which cell dis-adhesion typically plays a prominent role.

Phenotypes in breast and stomach cancer, related to *CDH1*, have been recently subject to increased attention to see what they have in common, in the hope that this knowledge will reflect on treatment outcome and cure rates. Indeed, it is the most modern attitude to clinically apparent cancers.

Subsequent to the clinical expediency there appear opportunities for prevention. Approximately less than 10% of all cancers, including breast and stomach cancer, are caused by inherited pathogenic genes. Carriers of these pathogenic variants suffer from hereditary cancer syndromes and present an accumulating risk of developing cancer during the course of their lives. The detection of the genetic risk, the germline presence of a DNA mutation in the *CDH1* gene, has many advanced clinical implications: management of genetic conditions in carriers such as awareness and knowledge about the potential genetic risk, sharing clinical information with family members, diagnostic tests, risk reduction procedures, coping with (additional) stress, and more are all aspects that need attention in the comprehensive care of people at increased risk for these deadly diseases. *CDH1* germline mutations increase the risk for diffuse gastric cancer, lobular breast cancer, or the combination of gastric and lobular breast cancer, and hereditary syndromes segregate families accordingly.

Awareness of the hereditary breast cancer syndrome changed process flow in the breast clinic. If the lobular subtype is present with positive family history for breast cancer and in the absence of the standard markers (*BRCA1/2, CHEK2*), there is an indication for sequencing *CDH1* and also *CTNNA1* in case of *CDH1* negativity. A personal or family history of multiple lobular breast cancers at a young age, even without diffuse gastric cancer, should prompt *CDH1* mutation screening. It is paramount to identify mutation carriers early, so that they can benefit from prophylactic gastrectomy before they develop symptomatic, highly lethal cancer. For hereditary diffuse gastric cancers that fit the clinical diagnosis of the syndrome but do not carry the *CDH1* or *CTNNA1* mutation, a new clinical entity of the "hereditary diffuse gastric cancer-like" group was created in 2020.

It is the group of Prof. Giovanni Corso that studied the *CDH1* mutation for many years now in a comprehensive way, and with success. Both cancers share germline mutations that result in a very special form of disease that is mutually related and expressed both in the breast and stomach. No other organs share this kind of molecular biology-driven characteristics in a significant way. As described above, research about these common pathways is extremely important to understand why two entirely different organs can create identical unique cancers.

When the picture becomes more complete, it is time to communicate the knowledge to the scientific and clinical world. Gradually, this knowledge should become available to all breast clinics and departments of gastroenterology in a way that carriers of the syndrome should not be missed. Indeed, lives of relatives are at stake.

Prof. Corso invited top scientific groups to create a thorough description of hereditary breast and gastric cancer, with epidemiology, care for mutation carriers, mutation variants, pathology phenotypes, endoscopic screening, surgery, and systemic treatments. The result is a truly multidisciplinary view on this interesting topic with attention to the implementation in clinical departments.

The question might be: Do the authors add useful knowledge to the approach of this hereditary syndrome so that mortality and incidence will decline?

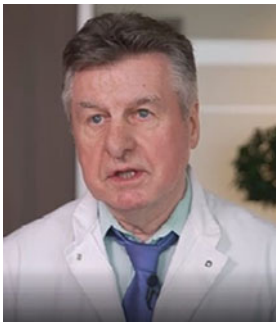
First, increased knowledge about the syndrome is always a step in the right direction. Basic research in epidemiology, genetics, pathology, diagnosis, treatments, and related disorders certainly will help understanding and empower cancer strategies.

Breast patients follow an entirely different clinical journey compared to patient with gastric diseases. And yet, individuals with *CDH1* mutations should be followed for both the breast and stomach. The only way to succeed proper care is to spread the information in both senology and gastroenterology where it can be picked up in proper patient care.

Early diagnosis is imperative but difficult to organize. Selection of individuals at high risk, the carriers of *CDH1* mutations, is necessary to increase success rates. Then there is the technology. For breast cancer, MRI-based imaging is the method of choice, while for diffuse gastric cancer only endoscopy with mucosal sampling is necessary. Tissue sampling of high quality is the cornerstone for diagnosis when suspicion is generated. Not only histological diagnosis and immunohistochemistry but also molecular profiling, including DNA mutation, is necessary in both syndromes.

Preventive measures in case of *CDH1* mutation are limited to prophylactic organ ablation. The role of surgery is therefore most important and new research defines when, how much, and how to correct function or esthetics. Drug repurposing as well as chemoprevention looks attractive additional but remote alternatives. Implementation in the future counts on contemporary research.

Altogether, although confined to stomach and breast hereditary *CDH1* syndromes, the field covered is extensive and ambitious in depth. A superb piece of science to build on for future generations.



European Cancer Prevention and
University of Hasselt
Hasselt, Belgium

Jaak Ph. Janssens

Contents

About the Editors

Giovanni Corso MD, PhD Breast surgeon at the European Institute of Oncology in Milan, Italy, and senior researcher at the University of Milan, Italy. Elected president of the European Cancer Prevention Organization (ECPO), and editor-in-chief of the European Journal of Cancer Prevention.

Paolo Veronesi MD Director of the Breast Surgery Division at the European Institute of Oncology in Milan, Italy, and full professor in surgery at the University of Milan, Italy. President of the Umberto Veronesi Foundation (FUV).

Franco Roviello MD Director of the Surgical Oncology Unit at the Hospital "Le Scotte" in Siena, Italy, and full professor in surgery at the University of Siena, Italy. President of the Italian Society of Surgical Oncology (SICO).

Part I

Epidemiology

Family History and the Risk of Breast and Gastric Cancer

1

Martino Bussa, Federica Turati, Rossella Bonzi, and Carlo La Vecchia

Abstract

Epidemiologists have used family history, usually of first-degree relatives, as a marker for genetic risk, knowing that family history reflects the consequences of genetic susceptibilities, shared environment, and common behaviors. The role of family history on breast and gastric cancer risk has been evaluated in multiple studies. As for breast cancer, informative, valid, and precise estimates of the role of family history derive from a reanalysis of individual data from 52 epidemiologic studies including over 58,000 women with breast cancer and 100,000 controls, which estimated an approximately twofold increased risk for women with family history; the risk increased with the number of affected relatives, decreased with age and was greater the younger the relatives were when their breast cancer was diagnosed. As for gastric cancer, a meta-analysis published in 2018 and based on 36 case-control and 4 cohort studies found a significant pooled relative risk of about 2; in line with that, a subsequent analysis based on individual participant data from 17 studies participating in the Stomach cancer Pooling (StoP) Project found an 80% increased risk in subject with at least on first-degree relative affected by gastric cancer.

1.1 Familial Breast Cancer

Worldwide, breast cancer is the most common cancer in women, accounting for around 12% of all female cancers [1]. Most breast cancers are sporadic and not associated with high penetrance gene mutations. A woman's risk of developing

M. Bussa · F. Turati (✉) · R. Bonzi · C. La Vecchia
Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy
e-mail: federica.turati@unimi.it

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_1

breast cancer is increased if she has a family history of the disease. In fact, family history is a widely recognized risk factor for breast cancer. About 20% of breast cancer patients have a family history of the disease and in one-fourth of these cases breast cancer appears to be inherited in an autosomal dominant fashion [2].

Hereditary breast cancer is associated with germline mutations in the BRCA1 and BRCA2 genes and is characterized by early onset and bilateral disease. Rare mutations in these susceptibility genes confer a 10–30 times higher risk of developing the disease compared to the general population [3]. BRCA1 and BRCA2 are high penetrance genes involved in DNA repair and DNA damage response [4, 5]. BRCA1 was located on chromosome 17q using linkage analysis in site-specific breast cancer families [6]. BRCA2 is localized on chromosome 13 [7]. Breast cancer risk is increased in women carrying a germline mutation in either BRCA1 or BRCA2. These mutations are responsible for the Hereditary Breast/Ovaric Cancer (HBOC) Syndrome. BRCA1 and BRCA2 mutations are inherited in an autosomal dominant fashion but behave as recessive alleles in somatic cells [8].

Disruptive mutations in the BRCA1 gene include an 11-base pair deletion, a 1-base pair insertion, a stop codon, a missense substitution, and a regulatory mutation [9].

The association between family history of breast cancer and breast cancer risk has been investigated in numerous epidemiologic studies. A comprehensive systematic review and meta-analysis published in 1997 and including 52 case-control and 33 cohort studies gave a pooled estimate of familial relative risk (RR) of 1.9 (95% confidence interval, CI 1.7–2.0) for any affected relative and 2.1 (95% CI 2.0–2.2) for an affected first-degree relative. In analyses by type of relative affected, the pooled RR were 1.8 (95% CI 1.6–2.0) for daughter, 2.0 (95% CI 1.8–2.1) for mother, 2.3 (95% CI 2.1–2.4) for sister, and 3.6 (95% CI 2.5–5.0) for mother and sister. Risks were increased in subjects under age 50 and when the relative had been diagnosed before age 50 [10].

After that review, Negri et al. [11] conducted in Italy a hospital-based case-control study on 2569 women aged less than 75 years with histologically confirmed incident breast cancer and 2588 control women admitted to hospitals for non-neoplastic condition. Compared with women with no history of breast cancer in first-degree relatives, the odds ratio (OR) for family history was 2.4 (95% CI 1.9–3.0), corresponding to an overall population attributable fraction (PAF) of approximately 7%. Women with only the mother affected had an OR of 2.26 (95% CI 1.6–3.2), those with only sister(s) an OR of 2.56 (95% CI 1.9–3.5), and those with both the mother and sister(s) affected an OR of 2.36 (95% CI 0.8–7.0). The PAF at all ages was 2.86% for mothers' history (95% CI 1.78–3.93), 3.15% for sisters' (95% CI 2.10–4.19), and 1.11 for other/combined (95% CI 0.46–1.76) [12].

In a population-based study of the Swedish Family-Cancer Database on 10.2 million individuals and more than 5500 familial breast cancers, Hemminki et al. [13] estimated familial standardized incidence ratios (SIR) of breast cancer of 1.79 by breast cancer in the mother only, 2.03 by breast cancer in a sister only, and 2.82 by breast cancer in both a mother and sister, and a PAF for familial breast cancer of 7.05% (3.61% for mother history, 3.01% for sister, 0.43% for both). The PAF values

Table 1.1 Risk ratios for breast cancer by number of first-degree relatives with a history of breast cancer, and for having a relative diagnosed with breast cancer at <40 years in strata of woman's age[a] from the Collaborative Group on Hormonal Factors in Breast Cancer analysis [14]

	Cases ($n = 58,209$)	Controls ($n = 101,986$)	Risk ratio (99%CI)[a]	Risk ratio for women <50 years (99%CI)	Risk ratio for women ≥50 years (99% CI)
Number of first-degree relatives with breast cancer					
None	50,713	94,548	1.0 (0.97–1.03)	Ref.	Ref.
1	6810	6998	1.80 (1.70–1.91)	2.14 (1.92–2.38)	1.65 (1.53–1.78)
2	603	404	2.93 (2.37–3.63)	3.84 (2.37–6.22)	2.61 (2.03–3.34)
3 or more	83	36	3.90 (2.03–7.49)	12.5 (1.70–85.16)	2.65 (1.29–5.46)
Relative's age at diagnosis of breast cancer <40 years[b]					
Woman's age (years)					
<40	125	41	5.7 (2.7–11.8)		
40–49	132	76	3.0 (1.8–4.9)		
50–59	94	107	2.0 (1.2–3.4)		
≥60	87	122	1.4 (0.9–2.1)		

CI: confidence interval

[a] Risk ratios are calculated as floating absolute risk (FAR, with FAR = 1.0 for women with no affected relative)

[b] The ref. category of the risk ratio is the group of women in the same age category with no affected relative

decreased by age when the daughter had a mother history of breast cancer but not when she had a sister history, and were not associated with the morphologic type of breast cancer.

In 2001, a re-analysis of individual data from 52 epidemiologic studies on familial breast cancer including 58,209 women with breast cancer and 101,986 control women confirmed the increased risk of breast cancer among women with a family history of the disease [14] (Table 1.1). Risk ratios for breast cancer were 1.80 (95% CI 1.69–1.91), 2.93 (95% CI 2.36–3.64), and 3.90 (95% CI 2.03–7.49) for one, two, and three or more affected first-degree relatives, respectively. The excess risk decreased with age and was greater the younger the relatives were when their breast cancer was diagnosed. Similar increased risks were observed according to the type of affected relative. In any case, most women who developed breast cancer did not have an affected first-degree relative. Authors estimated cumulative incidence of breast cancer up to age 50 of 1.7%, 3.7%, and 8.0% for women with zero, one, or two affected first-degree relatives, respectively, in more-developed countries; corresponding estimates for incidence up to age 80 were 7.8%, 13.3%, and 21.1%, and for death from breast cancer up to age 80 were 2.3%, 4.2%, and 7.6%.

More recently, Kuchenbaecker et al. [15] estimated cumulative risks of breast cancer for BRCA1 and BRCA2 mutation carriers using data from a prospective cohort. The cumulative risk of developing breast cancer by age 80 years was 72% for BRCA1 mutation carriers and 69% for BRCA2 mutation carriers, respectively. The cumulative risk to age 50 years were higher for BRCA1 carriers. In addition, breast cancer risk was higher if BRCA1 mutations were located outside vs within the regions bounded by positions c.2282 to c.4071 (hazard ratio, HR = 1.46; 95% CI 1.11–1.93).

Research has made significant further efforts to identify other susceptibility genes for breast cancer that also operate in the DNA damage response. TP53 is a tumor suppressor gene that causes Li Fraumeni syndrome [16]. TP53 mutation carriers are predisposed to a variety of different tumors, including sarcomas, brain tumors, breast cancers, and adrenocortical carcinomas, diagnosed before the age of 45 years [17]. In 265 families with a germline TP53 mutation or affected with Li-Fraumeni syndrome, breast cancer was the most frequent malignancy (30.6%), followed by soft tissue sarcoma (17.8%), brain tumor (14%), and adrenocortical carcinoma (6.5%). All of the breast cancers were in female TP53 mutation carriers [18].

The ATM gene encodes a protein kinase with an important role in DNA repair [19]. Biallelic mutations in the ATM gene cause ataxia-telangiectasia, a rare autosomal recessive neurological disorder characterized by cancer predisposition, in particular lymphomas and leukemia [20]. By contrast, heterozygous female ATM mutation carriers are at elevated risk of breast cancer [21]. Thompson et al. [22] observed a significant excess of female breast cancer in heterozygous female ATM mutation carriers (RR = 2.23, 95% CI 1.16–4.28) compared with the general population, but the RR was 4.94 (95%IC, 1.90–12.9) in women younger than age 50 years. A meta-analysis published in 2016 estimated a pooled RR of 3.0 (95% CI 2.1–4.5) of breast cancer in female obligate ATM heterozygotes [23].

Another gene that confers susceptibility to breast cancer is the CHEK2 gene, which encodes a kinase protein involved in DNA repair [24]. The CHEK2*1100*del*C mutation confers an about twofold increased breast cancer risk in women and a tenfold increased risk in men. This truncating mutation was found in 5.1% of individuals with breast cancer from families without BRCA1 or BRCA2 mutation [25]. By contrast, its frequency is of 1.1% in the healthy population. In a large case-control study conducted in Poland a truncating CHECK mutation (1100delC) was present in 227 (3%) of 7496 women with breast cancer and in 37 (0.8%) of 4346 controls (OR = 3.6, 95% CI 2.6-5.1). The OR was higher for women with a first- or second-degree relative with breast cancer (OR = 5.0, 95% IC 3.3–7.6) than for women with no family history (OR = 3.3; 95% CI 2.3–4.7) [26]. The authors estimated the lifetime risk of breast cancer for CHEK2*1100delC carriers to be 20% for women with no affected relative. Female homozygotes for the CHEK2*1100delC have a risk of breast cancer increased more than twice the risk of heterozygous carriers [27].

In conclusion, epidemiological evidence indicates an approximately twofold increased breast cancer risk associated with family history of the disease. In any case, most women who develop breast cancer do not have an affected relative. Still,

in high-income countries women with a first-degree relative with breast cancer have an over 10% lifetime cumulative risk of developing breast cancer [14].

1.2 Familial Gastric Cancer

Gastric cancer is a global health problem, with more than one million incident cases worldwide each year, ranking fifth for incidence and fourth for mortality globally in 2020 [1]. The classification of Lauren distinguishes two main types of gastric carcinoma, diffuse gastric cancer and intestinal-type gastric cancer, which display different molecular, epidemiologic, and morphologic features [28].

Although gastric cancer is usually sporadic, it occurs more frequently among close relatives of affected patients than in the general population. Familial aggregation is observed in about 10% of cases [29, 30]. The importance of family history, a proxy of hereditary and genetic factors, as a risk factor for gastric cancer has been evaluated in several studies, mostly case-control studies [31]. In general, these studies gave estimates of the familial RR of gastric cancer ranging from 1.5 to 3, with however a few studies from Asia, where the rate of the disease is notoriously higher compared with Western countries, providing dramatically elevated RR, over 6–7. Differences in the strength of the association across studies conducted in various populations may be in part attributed to their different baseline characteristics, lifestyle habits, and rates of gastric cancer.

Among the earliest studies, a hospital-based case-control study in Italy studied the familial occurrence of cancer in 154 patients with gastric cancer registered in 1986 and 1987 and in 154 controls matched by age and sex by tracing a careful genealogical tree of first-degree relatives [29]. Thirty first-degree relatives with gastric cancer were reported in case families (3.3%) versus 15 in control families (1.5%), for a corresponding OR of 3.14 ($p < 0.01$). The excess of gastric cancer was more marked in siblings (OR = 4.33, $p < 0.02$) than in parents (OR = 1.61, not significant). No significant excess of other types of cancers in case families was observed. In another Italian hospital-based case-control study conducted in 1985–1991 and including 628 cases and 1776 controls, the prevalence of family history of gastric cancer was 12.6% among cases and 4.9% among controls. The corresponding OR adjusted for age, sex, area of residence, education, and number of siblings was 2.6 (95% CI 1.8–3.6), being similar for having affected parents (OR = 2.4, 95% CI 1.7–3.4) and affected siblings (OR = 2.5, 95% CI 1.3–4.6), and directly related with the number of first-degree relatives affected. In terms of PAF, approximately 8% of gastric cancers in that population were related to the familial component [30].

Several case-control studies were published thereafter. Among the larger ones, a study from Poland [32] showed an over threefold increase in risk for a history of gastric cancer in first-degree relative (OR = 3.5, 95% CI 2.0–6.2) based on 464 cases and 480 controls. The OR for family history was 6.6 for affected parents (95% CI 4.20–10.40) and 10.1 for affected siblings (95% CI 6.10–16.82) in a hospital-based case-control study carried out in Turkey with 1240 cases and 1240 controls [33, 34], and 3.67 (95% CI 2.01–6.71) in a case-control study from Spain with 404 cases and

404 controls [35]. In a large population-based case-control study conducted in Japan (1400 cases, 13,467 controls) the OR for family history was greater in the younger age group (≤43 years) than in the older age group (>43 years), i.e., 6.3 (95% CI 4.1–9.9) and 4.4 (95% CI 3.9–5.0), respectively [36].

Only a few prospective cohort studies, mainly from Asia, evaluated family history as a risk factor for gastric cancer, with mixed results. In a large cohort study, in which 19,028 individuals from the Japanese Public Health Center cohort II were followed-up from 1993 to 2009, gastric cancer history in first-degree relatives was associated with an increased risk gastric cancer with a HR of 1.30 (95% CI 1.25–1.35), based on 412 incident cases [37]. In a Japanese case-control study nested in a cohort, family history of gastric cancer in first-degree relatives was associated with an increased risk of the disease in women, but not in men, after controlling for *Helicobacter pylori* infection and other confounding variables, with RR of 1.73 (95% CI 0.82–3.65) and 0.89 (95% CI 0.40–1.97), respectively [38]. Only one cohort study was conducted in a Western population, specifically in Finland. A total of 307 incident gastric cancer cases among 20,720 male smokers were identified during the follow-up period. Gastric cancer history in any first-degree relatives was associated with an approximately 1.5-fold increased gastric cancer, after adjustment for age, number of siblings, body mass index, smoking, alcohol, education, and fruit and vegetable intake (HR = 1.56, 95% CI 1.15–2.12) [39].

In 2018, a meta-analysis including 40 observational studies was published. The pooled RR for family history of gastric cancer was 2.31 (95% CI 1.99–2.68) from all studies ($n = 40$), 2.56 (95% CI 2.12–3.10) from case-control studies ($n = 36$), and 1.30 (95% CI 1.26–1.34) from cohorts ($n = 4$). Family history of gastric cancer was significantly associated with non-cardia (pooled RR = 1.97, 95% CI 1.72–2.25), but not with cardia gastric cancer (pooled RR = 1.46, 95% CI 0.89–2.39). The association appeared stronger for family history of gastric cancer in siblings (pooled RR = 2.84, 95% CI 1.91–4.24) than in parents (pooled RR = 2.16, 95% CI 1.68–2.76) [39].

More recently, the association between family history of gastric cancer and gastric cancer risk was investigated within a large consortium of epidemiological studies on gastric cancer, the Stomach cancer Pooling (StoP) Project [40]. The analysis was based on 5949 cases of gastric cancer and 12,776 controls from 17 case-control studies from 11 countries. Most studies were conducted in Europe (82.3% of the controls and 77.9% of the cases). Family history of gastric cancer resulted directly related with gastric cancer with a pooled OR of 1.8 (95% CI 1.64–2.04), in the absence of material heterogeneity among studies ($I^2 = 6.1\%$, $P_{\text{heterogeneity}} = 0.838$) (Fig. 1.1). The pooled OR was higher for having affected siblings than affected parents (OR =1.6, 95% CI 1.20–2.05, and OR = 1.5,95% CI 1.28–1.80, respectively). There were no significant differences among subgroups by sex, age, geographic area, or study period. In that pooled investigation, family history has a greater pooled OR on non-cardia (OR = 1.82, 95% CI 1.59–2.05) than cardia gastric cancer (OR = 1.38, 95% CI 0.98–1.77). The occurrence of non-cardia gastric cancer is mainly attributed to

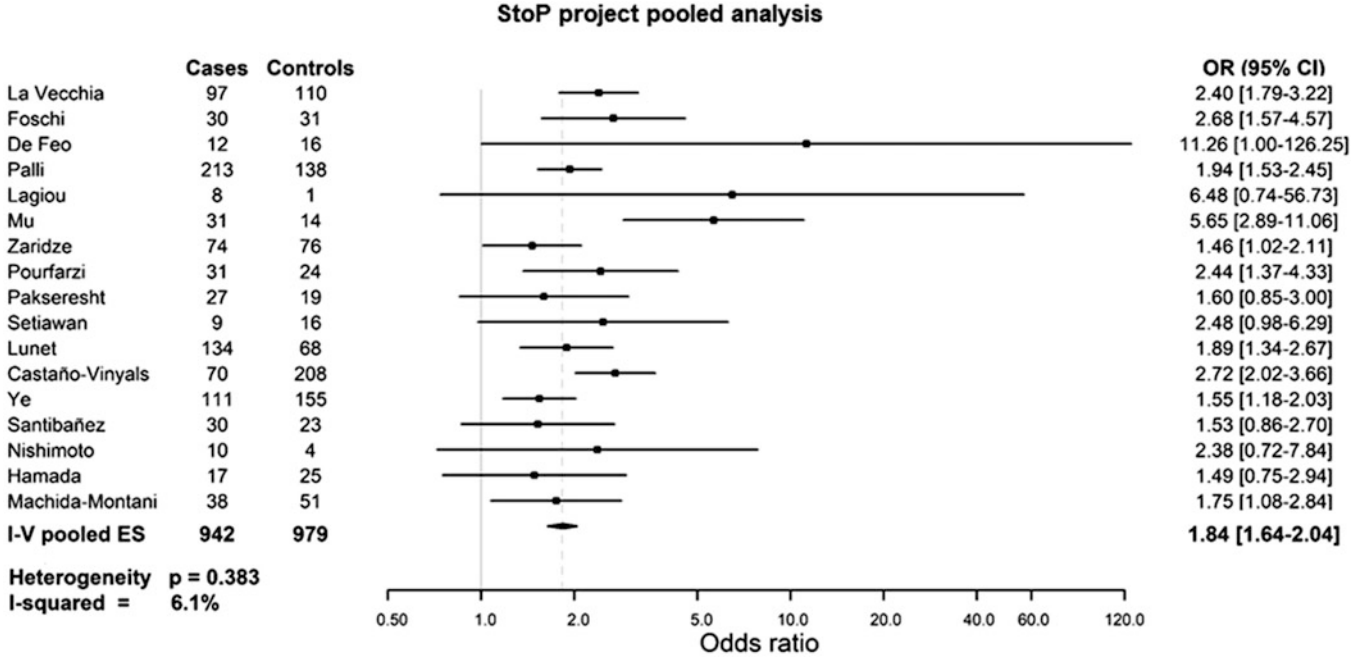


Fig. 1.1 Study-specific and pooled adjusted odds ratios (OR) with corresponding 95% confidence intervals (CI) of gastric cancer for family history of gastric cancer in first-degree relatives in the StoP Project [40]

Helicobacter pylori atrophic gastritis and, therefore, is more likely associated with familial clustering [41]. On the other hand, cardia gastric cancer is more likely related to lifestyle factors, such as obesity, gastroesophageal reflux, western diet, and tobacco smoking [40–45].

The familial aggregation of gastric cancer is due to a complex interaction between genetic inheritance and environmental and lifestyle factors. It is known that between 10% and 20% of people who develop gastric cancer have family history, but only part of this can be attributed to a hereditary syndrome. The estimates based on family history involve both genetic and shared environment factors, specifically *H. pylori*, which is the primary risk factor in gastric carcinogenesis and tends to cluster in families [46]. However, in the pooled analysis within the Stop Project the association with family history of gastric cancer was similar in subgroups defined by *H. pylori* infection [40].

A combination of linkage and mutation analysis identified in an extended New Zealand Maori family with early onset diffuse gastric cancer the gene for the cell-to-cell adhesion protein *E-cadherin* as a cancer-susceptibility gene [47]. Epithelial cadherin is a cell adhesion protein predominantly expressed in epithelial tissue. This cell adhesion molecule plays an important role in establishing cell polarity and maintaining epithelial tissue morphology. *E-cadherin* molecules are generally localized at the basolateral surface of the cell, in a region of cell-cell contact that is known as zonula adherences junctions [48, 49]. E-cadherin is encoded by CDH1 that maps to chromosome 16q22.1. Sequencing of the *E-cadherin* gene revealed a G T nucleotide substitution (position 1008) of 7 exon, leading to a truncated gene product. To confirm the role of *E-cadherin* in hereditary gastric cancer susceptibility, the authors identified *E-cadherin* germline truncating mutations in two other families of Maori ethnicity with early-onset diffuse gastric cancer. This first genetic linkage study demonstrated the role of *E-cadherin* germline mutations in familial diffuse gastric cancer [47]. Shortly afterward, *E-cadherin* germline truncating mutations were detected in three families of European origin with familial diffuse gastric cancer [50] and subsequently, *E-cadherin* germline mutations have been identified in similar families from several countries reinforcing the role of *CDH1* in susceptibility to diffuse gastric cancer in other populations. The first *CDH1* germline missense mutation has been described in an Italian family with hereditary diffuse gastric cancer [51]. All of these families have diffuse-type gastric cancer and *CDH1* germline mutations have not been described in eight families of European origin with intestinal gastric cancer [50]. This specificity of tumor type has led to the identification of this new familial cancer syndrome, designated Hereditary Diffuse Gastric Cancer (HDGC), characterized by high prevalence of diffuse gastric cancer and lobular breast cancer [52, 53]. Heterozygous carriers of a *E-cadherin* germline mutation have a high lifetime risk of developing gastric cancer and lobular breast cancer. The cumulative risk of gastric cancer in *CDH1* mutation carriers increases steadily from early adulthood. The estimated cumulative risk of diffuse gastric cancer in mutation carriers by age 80 years was 67% for men (95% CI 39–99%) and 83% for women (95% CI, 58–99) [54]. In 1999, specific clinical criteria have been set to select individuals for *CDH1* genetic screening. Using the first guidelines

established in 1999 the detection rate of *CDH1* mutations was approximately 40% in individuals fulfilling the clinical criteria [55]. However, the guidelines were subsequently revised given that *CDH1* germline mutations were also identified in individuals who did not meet testing criteria. Hansford and colleagues [56] reported in the largest series of *CDH1* mutations carriers that the cumulative risk of diffuse gastric cancer by age 80 years was 70% (95% CI 59–80%) for men and 56% (95% CI 44–69%) for women, whereas breast cancer lifetime risk for women was 42% (95% CI 23–68%). HDGC caused by germline *CDH1* mutations is an autosomal dominant cancer syndrome.

Different patterns of *CDH1* germline mutations have been described as missense, non-sense, deletion, and splice-site. Insertions are less frequently described, constituting about 10% of all *CDH1* mutations. Corso and colleagues [57] verified that the predominant mutation type varies across geographical regions. Deletions are more frequent in Europe (34%), splice-site in America (48%), missense in Asia (68%), and non-sense in Oceania (78%). There are few other genes which are involved in HDGC predisposition, including *CTNNA1*. Like *CDH1*, *CTNNA1* is involved in intercellular adhesion. Germline *CTNNA1* alterations cause HDGC on occasion and should be considered in screening of prospective families [53].

It is therefore important to take into account the presence of a gastric cancer history in first-degree relatives to carry out gastric cancer early diagnosis.

References

1. Sung H, Ferlay J, Siegel RL et al (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71(3): 209–249. https://doi.org/10.3322/caac.21660
2. Andersen TI (1996) Genetic heterogeneity in breast cancer susceptibility. Acta Oncol 35(4): 407–410. https://doi.org/10.3109/02841869609109913
3. Christinat A, Pagani O (2013) Practical aspects of genetic counseling in breast cancer: lights and shadows. Breast 22(4):375–382. https://doi.org/10.1016/j.breast.2013.04.006
4. Welcsh PL, King MC (2001) BRCA1 and BRCA2 and the genetics of breast and ovarian cancer. Hum Mol Genet 10(7):705–713. https://doi.org/10.1093/hmg/10.7.705
5. Apostolou P, Fostira F (2013) Hereditary breast cancer: the era of new susceptibility genes. Biomed Res Int 2013:747318. https://doi.org/10.1155/2013/747318
6. Hall JM, Lee MK, Newman B et al (1990) Linkage of early-onset familial breast cancer to chromosome 17q21. Science 250(4988):1684–1689. https://doi.org/10.1126/science.2270482
7. Wooster R, Bignell G, Lancaster J et al (1995) Identification of the breast cancer susceptibility gene BRCA2. Nature 378(6559):789–792. https://doi.org/10.1038/378789a0
8. Shiovitz S, Korde LA (2015) Genetics of breast cancer: a topic in evolution. Ann Oncol 26(7): 1291–1299. https://doi.org/10.1093/annonc/mdv022
9. Miki Y, Swensen J, Shattuck-Eidens D et al (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266(5182):66–71. https://doi.org/10.1126/science.7545954
10. Pharoah PD, Day NE, Duffy S et al (1997) Family history and the risk of breast cancer: a systematic review and meta-analysis. Int J Cancer 71(5):800–809. https://doi.org/10.1002/(sici)1097-0215(19970529)71:5<800::aid-ijc18>3.0.co;2-b

11. Negri E, Braga C, La Vecchia C et al (1997) Family history of cancer and risk of breast cancer. Int J Cancer 72(5):735–738. https://doi.org/10.1002/(sici)1097-0215(19970904)72:5<735::aid-ijc5>3.0.co;2-t
12. Pelucchi C, Negri E, Tavani A et al (2002) Attributable risk for familial breast cancer. Int J Cancer 102(5):548–549. https://doi.org/10.1002/ijc.10760
13. Hemminki K, Granstrom C, Czene K (2002) Attributable risks for familial breast cancer by proband status and morphology: a nationwide epidemiologic study from Sweden. Int J Cancer 100(2):214–219. https://doi.org/10.1002/ijc.10467
14. Collaborative Group on Hormonal Factors in Breast C (2001) Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. Lancet 358(9291):1389–1399. https://doi.org/10.1016/S0140-6736(01)06524-2
15. Kuchenbaecker KB, Hopper JL, Barnes DR et al (2017) Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. JAMA 317(23):2402–2416. https://doi.org/10.1001/jama.2017.7112
16. Mavaddat N, Antoniou AC, Easton DF et al (2010) Genetic susceptibility to breast cancer. Mol Oncol 4(3):174–191. https://doi.org/10.1016/j.molonc.2010.04.011
17. Gonzalez KD, Noltner KA, Buzin CH et al (2009) Beyond Li Fraumeni syndrome: clinical characteristics of families with p53 germline mutations. J Clin Oncol 27(8):1250–1256. https://doi.org/10.1200/JCO.2008.16.6959
18. Olivier M, Goldgar DE, Sodha N et al (2003) Li-Fraumeni and related syndromes: correlation between tumor type, family structure, and TP53 genotype. Cancer Res 63(20):6643–6650
19. Torabi Dalivandan S, Plummer J, Gayther SA (2021) Risks and function of breast cancer susceptibility alleles. Cancers (Basel) 13(16). https://doi.org/10.3390/cancers13163953
20. Turati F, Negri E, La Vecchia C (2014) Family history and the risk of cancer: genetic factors influencing multiple cancer sites. Expert Rev Anticancer Ther 14(1):1–4. https://doi.org/10.1586/14737140.2014.863713
21. Stratton MR, Rahman N (2008) The emerging landscape of breast cancer susceptibility. Nat Genet 40(1):17–22. https://doi.org/10.1038/ng.2007.53
22. Thompson D, Duedal S, Kirner J et al (2005) Cancer risks and mortality in heterozygous ATM mutation carriers. J Natl Cancer Inst 97(11):813–822. https://doi.org/10.1093/jnci/dji141
23. van Os NJ, Roeleveld N, Weemaes CM et al (2016) Health risks for ataxia-telangiectasia mutated heterozygotes: a systematic review, meta-analysis and evidence-based guideline. Clin Genet 90(2):105–117. https://doi.org/10.1111/cge.12710
24. Nevanlinna H, Bartek J (2006) The CHEK2 gene and inherited breast cancer susceptibility. Oncogene 25(43):5912–5919. https://doi.org/10.1038/sj.onc.1209877
25. Meijers-Heijboer H, van den Ouweland A, Klijn J et al (2002) Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. Nat Genet 31(1):55–59. https://doi.org/10.1038/ng879
26. Cybulski C, Wokolorczyk D, Jakubowska A et al (2011) Risk of breast cancer in women with a CHEK2 mutation with and without a family history of breast cancer. J Clin Oncol 29(28):3747–3752. https://doi.org/10.1200/JCO.2010.34.0778
27. Adank MA, Jonker MA, Kluijt I et al (2011) CHEK2*1100delC homozygosity is associated with a high breast cancer risk in women. J Med Genet 48(12):860–863. https://doi.org/10.1136/jmedgenet-2011-100380
28. Lauren P (1965) The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 64:31–49. https://doi.org/10.1111/apm.1965.64.1.31
29. Zanghieri G, Di Gregorio C, Sacchetti C et al (1990) Familial occurrence of gastric cancer in the 2-year experience of a population-based registry. Cancer 66(9):2047–2051. https://doi.org/10.1002/1097-0142(19901101)66:9<2047::aid-cncr2820660934>3.0.co;2-g

30. La Vecchia C, Negri E, Franceschi S et al (1992) Family history and the risk of stomach and colorectal cancer. Cancer 70(1):50–55. https://doi.org/10.1002/1097-0142(19920701)70:1<50::aid-cncr2820700109>3.0.co;2-i
31. Yaghoobi M, Bijarchi R, Narod SA (2010) Family history and the risk of gastric cancer. Br J Cancer 102(2):237–242. https://doi.org/10.1038/sj.bjc.6605380
32. Lissowska J, Groves FD, Sobin LH et al (1999) Family history and risk of stomach cancer in Warsaw. Poland Eur J Cancer Prev 8(3):223–227. https://doi.org/10.1097/00008469-199906000-00010
33. Bakir T, Can G, Erkul S et al (2000) Stomach cancer history in the siblings of patients with gastric carcinoma. Eur J Cancer Prev 9(6):401–408. https://doi.org/10.1097/00008469-200012000-00005
34. Bakir T, Can G, Siviloglu C et al (2003) Gastric cancer and other organ cancer history in the parents of patients with gastric cancer. Eur J Cancer Prev 12(3):183–189. https://doi.org/10.1097/00008469-200306000-00003
35. Garcia-Gonzalez MA, Lanas A, Quintero E et al (2007) Gastric cancer susceptibility is not linked to pro-and anti-inflammatory cytokine gene polymorphisms in whites: a nationwide multicenter study in Spain. Am J Gastroenterol 102(9):1878–1892. https://doi.org/10.1111/j.1572-0241.2007.01423.x
36. Eto K, Ohyama S, Yamaguchi T et al (2006) Familial clustering in subgroups of gastric cancer stratified by histology, age group and location. Eur J Surg Oncol 32(7):743–748. https://doi.org/10.1016/j.ejso.2006.04.005
37. Charvat H, Sasazuki S, Inoue M et al (2016) Prediction of the 10-year probability of gastric cancer occurrence in the Japanese population: the JPHC study cohort II. Int J Cancer 138(2):320–331. https://doi.org/10.1002/ijc.29705
38. Yatsuya H, Toyoshima H, Tamakoshi A et al (2004) Individual and joint impact of family history and Helicobacter pylori infection on the risk of stomach cancer: a nested case-control study. Br J Cancer 91(5):929–934. https://doi.org/10.1038/sj.bjc.6602067
39. Song M, Camargo MC, Weinstein SJ et al (2018) Family history of cancer in first-degree relatives and risk of gastric cancer and its precursors in a Western population. Gastric Cancer 21(5):729–737. https://doi.org/10.1007/s10120-018-0807-0
40. Vitelli-Storelli F, Rubin-Garcia M, Pelucchi C et al (2021) Family history and gastric cancer risk: a pooled investigation in the stomach cancer pooling (STOP). Project Consortium Cancers (Basel) 13(15). https://doi.org/10.3390/cancers13153844
41. Kharazmi E, Babaei M, Fallah M et al (2018) Importance of tumor location and histology in familial risk of upper gastrointestinal cancers: a nationwide cohort study. Clin Epidemiol 10:1169–1179. https://doi.org/10.2147/CLEP.S168152
42. Bahmanyar S, Ye W (2006) Dietary patterns and risk of squamous-cell carcinoma and adenocarcinoma of the esophagus and adenocarcinoma of the gastric cardia: a population-based case-control study in Sweden. Nutr Cancer 54(2):171–178. https://doi.org/10.1207/s15327914nc5402_3
43. Gonzalez CA, Pera G, Agudo A et al (2003) Smoking and the risk of gastric cancer in the European prospective investigation into cancer and nutrition (EPIC). Int J Cancer 107(4):629–634. https://doi.org/10.1002/ijc.11426
44. Chen Y, Liu L, Wang X et al (2013) Body mass index and risk of gastric cancer: a meta-analysis of a population with more than ten million from 24 prospective studies. Cancer Epidemiol Biomark Prev 22(8):1395–1408. https://doi.org/10.1158/1055-9965.EPI-13-0042
45. Lin XJ, Wang CP, Liu XD et al (2014) Body mass index and risk of gastric cancer: a meta-analysis. Jpn J Clin Oncol 44(9):783–791. https://doi.org/10.1093/jjco/hyu082
46. Lyons K, Le LC, Pham YT et al (2019) Gastric cancer: epidemiology, biology, and prevention: a mini review. Eur J Cancer Prev 28(5):397–412. https://doi.org/10.1097/CEJ.0000000000000480
47. Guilford P, Hopkins J, Harraway J et al (1998) E-cadherin germline mutations in familial gastric cancer. Nature 392(6674):402–405. https://doi.org/10.1038/32918

48. Grunwald GB (1993) The structural and functional analysis of cadherin calcium-dependent cell adhesion molecules. Curr Opin Cell Biol 5(5):797–805. https://doi.org/10.1016/0955-0674(93)90028-o
49. Takeichi M (1991) Cadherin cell adhesion receptors as a morphogenetic regulator. Science 251(5000):1451–1455. https://doi.org/10.1126/science.2006419
50. Gayther SA, Gorringe KL, Ramus SJ et al (1998) Identification of germ-line E-cadherin mutations in gastric cancer families of European origin. Cancer Res 58(18):4086–4089
51. Roviello F, Corso G, Pedrazzani C et al (2007) Hereditary diffuse gastric cancer and E-cadherin: description of the first germline mutation in an Italian family. Eur J Surg Oncol 33(4):448–451. https://doi.org/10.1016/j.ejso.2006.10.028
52. Caldas C, Carneiro F, Lynch HT et al (1999) Familial gastric cancer: overview and guidelines for management. J Med Genet 36(12):873–880
53. Blair VR, McLeod M, Carneiro F et al (2020) Hereditary diffuse gastric cancer: updated clinical practice guidelines. Lancet Oncol 21(8):e386–ee97. https://doi.org/10.1016/S1470-2045(20)30219-9
54. Pharoah PD, Guilford P, Caldas C et al (2001) Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. Gastroenterology 121(6):1348–1353. https://doi.org/10.1053/gast.2001.29611
55. Kaurah P, MacMillan A, Boyd N et al (2007) Founder and recurrent CDH1 mutations in families with hereditary diffuse gastric cancer. JAMA 297(21):2360–2372. https://doi.org/10.1001/jama.297.21.2360
56. Hansford S, Kaurah P, Li-Chang H et al (2015) Hereditary diffuse gastric cancer syndrome: CDH1 mutations and beyond. JAMA Oncol 1(1):23–32. https://doi.org/10.1001/jamaoncol.2014.168
57. Corso G, Corso F, Bellerba F et al (2021) Geographical distribution of E-cadherin germline mutations in the context of diffuse gastric cancer: a systematic review. Cancers (Basel) 13(6). https://doi.org/10.3390/cancers13061269

Worldwide *CDH1* Germline Mutation Frequency

2

Giulia Massari, Valentina Tagliaferri, and Giovanni Corso

Abstract

E-cadherin (*CDH1* gene) germline mutations are associated with the development of the autosomal cancer syndrome known as hereditary diffuse gastric cancer. Different patterns of *CDH1* germline mutations have been described as truncating, deletion, insertion, Splice-site, non-sense, silence, and at last, missense alterations. The frequency of the different E-cadherin germline mutations in countries with different incidence rates for gastric carcinoma has been reported as extremely variable. In particular, the missense variant frequency seems to be higher in high-incidence areas of gastric cancer, when compared with non-missense mutations. In this chapter, we described the worldwide frequency of *CDH1* germline mutations in gastric cancers coming from different geographical areas.

Parts of this chapter are based on the open access publication Corso, G.; Corso, F.; Bellerba, F.; Carneiro, P.; Seixas, S.; Cioffi, A.; La Vecchia, C.; Magnoni, F.; Bonanni, B.; Veronesi, P.; et al. Geographical Distribution of E-cadherin Germline Mutations in the Context of Diffuse Gastric Cancer: A Systematic Review. Cancers 2021, 13, 1269. https://doi.org/10.3390/cancers13061269

G. Massari · V. Tagliaferri
Division of Breast Surgery, European Institute of Oncology, IRCCS, Milan, Italy

G. Corso (✉)
Division of Breast Surgery, European Institute of Oncology, IRCCS, Milan, Italy

Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy
e-mail: giovanni.corso@ieo.it

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_2

2.1 Introduction

The first description of *CDH1* germline mutations was reported in Māori kindred and families with diffuse gastric cancer (DGC) and lobular breast cancer (LBC) aggregation [1]. In 1999, the International Gastric Cancer Linkage Consortium (IGCLC) defined the hereditary diffuse gastric cancer (HDGC) syndrome and established clinical criteria for *CDH1* genetic screening of individuals and families at risk [2]. Using those first guidelines, the detection rate of *CDH1* mutations was approximately 40% in individuals fulfilling the clinical criteria [3]. However, the guidelines were subsequently revised given that *CDH1* germline mutations were also identified in individuals who did not meet testing criteria [4–6]. Hansford and colleagues reported that in individuals meeting the IGCLC 2010 criteria and with *CDH1* germline mutation [5], the cumulative lifetime GC risk at 80 years of age was 70% (95% CI, 59–80%) for males and 56% (95% CI, 44–69%) for females, whereas breast cancer lifetime risk for females was 42% (95% CI, 23–68%) [7].

To date, more than 155 *CDH1* mutations affecting the entire coding sequence and functional domains of E-cadherin have been identified in the context of HDGC [5, 7]. Whereas the majority of HDGC patients display *CDH1* truncating mutations that induce a deleterious effect and are thus a bona fide DGC cause, around 20% harbor mutations of the missense type, which represent a major clinical challenge [5]. Indeed, missense variants are difficult to assess phenotypically, thus leading to critical issues concerning genetic counseling and clinical management. Further, their incomplete penetrance masks their identification and classification, contributing to variant dissemination among populations [8]. Importantly, failure to incorporate information and ascertain pathogenicity of missense variants perpetuates misestimating of *CDH1* penetrance and the diagnostic dilemma surrounding affected families. In an era of high-throughput genome sequencing and multiplex gene panel testing, this problem is becoming unwieldy with the identification of an increasing number of variants of unknown significance (VUS), not only in disease but also in individuals without a family history of GC [9, 10]. This, along with the fact that the majority of cancer screening programs do not recommend *CDH1* testing in the absence of specific clinical criteria, urges the need to streamline *CDH1* screening. More so, there is a lack of systematic results regarding *CDH1* genetic screening across countries, creating a void in terms of mutation geographic distribution [11]. This has prompted us to perform a comprehensive evaluation of germline mutations associated with HDGC, which may explain the large variability in GC epidemiology and provide insights to define priorities for effective screening and improved management.

2.2 Gastric Cancer Epidemiology

In the first half of the past century, GC was the most common cause of cancer-related deaths worldwide [12]. Although steadily declining, in 2017 GC remained the third cause of cancer-related deaths, after lung and colorectum, with almost over 850,000

deaths globally [13]. It was also the third cause of years of life lost (YLL), after lung and liver cancer [14].

The epidemiology of stomach cancer has important geographical heterogeneity, and its incidence can vary fivefold to tenfold between high-risk and low-risk countries [13]. Part of this geographical diversities correlates with *H. pylori* infection rates across populations; however, other environmental factors also contribute to the GC risk. Cigarette smoking has been shown to be a risk [15]. Salt and salt-preserved foods increase the risk of stomach cancer. In general, GC is more common among males, which might be due to the higher prevalence of risk factors, such as smoking, or hormonal factors contributing to this difference. Although survival rates have generally improved over the past several decades, the overall prognosis remains poor [16]. The 5-year survival rate is about 20%, with the notable exceptions of 65% in Japan [17] and 71.5% in South Korea [18], where population screening has improved the early diagnosis of gastric tumors at early stages.

A total of 1.22 million incident cases were estimated, with the highest incidence reported in Asia Pacific and East Asia, of which almost 50% were in China [14]. In the European Union, about 100,000 GC-related deaths were predicted for 2020 [19].

Almost two-thirds of GC cases occur in developing countries with 42% in China alone [20]. In fact, the geographical distribution of GC is widely heterogeneous, with high-risk areas including East Asia (China, Japan, and Korea), Eastern Europe, and parts of Central and South America [12, 21–24]. Incidence rates are lower (<10 per 100,000 in men) in Southern Asia, North and East Africa, North America, Australia, and New Zealand [21]. Eastern Europe is the highest European risk area for GC with an incidence of 70,000 per year (Belarus area) [25]. Portugal and Italy also represent relevant European areas for stomach cancer prevalence, with incidence reports around 41,100 and 33,400 per year, respectively [26].

A recent report from the *GBD 2017 Stomach Cancer Collaborators* reported a detailed world map of age-standardized incidence rates of stomach cancer in 2017 [13]. The highest age-standardized incidence rate was seen in the high-income Asia Pacific region (29.5 per 100,000 population), particularly in Japan and South Korea, and east Asia (28.6 per 100,000 population). In east Asia, China alone had nearly half of the global incident cases in 2017 (562,000). Eastern Europe (17.7) and Andean Latin America (16.6) regions had the next highest age-standardized incidence rates. Mongolia (35.6) and Afghanistan (32.8) had the overall highest age-standardized incidence rates. The lowest incidence rates were seen in southern and eastern sub-Saharan Africa and high-income North America. East Asia had the highest age-standardized death rate (18.7), followed by Andean Latin America (17.1) and central Asia (14.3). The high-income Asia Pacific region, which ranked first in age-standardized incidence rate, had the fourth highest age-standardized death rate. The two countries with the highest age-standardized incidence rate also had the highest age-standardized death rates: Mongolia (37.6) and Afghanistan (33.6). The lowest age-standardized death rates were seen in high-income North America and Australasia.

In general, widespread decline in GC incidence and mortality have been mainly associated with the implementation of *Helicobacter pylori* eradication programs,

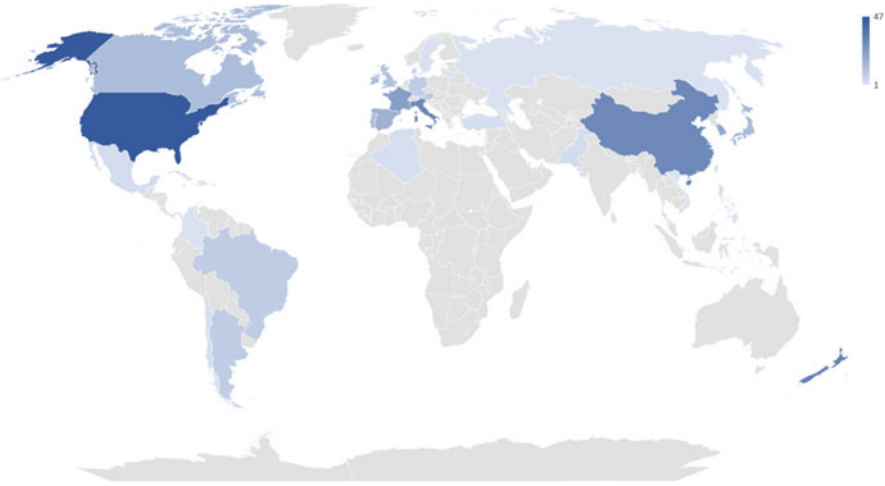


Fig. 2.1 Worldwide distribution of germline *CDH1* mutations. USA and New Zealand have reported the highest concentration of germline *CDH1* variants, followed by China, Italy, and France

along with socio-economic improvements and advances in diet and food preservation. In contrast, cancer of the gastric cardia has increased in several high-income countries due to the increase in overweight and obesity, known etiological factors of gastroesophageal reflux [27].

Although environmental risk factors account for variations in incidence and mortality rates worldwide, family history is a major risk factor for gastric cancer [28]. A number of genetic loci have been associated with GC risk, which may directly impact disease progression or interact with environmental factors in the causal pathway [12, 29].

GC presents familial aggregation in about 10%, and only 3% show a clear inherited cancer predisposition, so-called "hereditary", associated with a documented germline mutation. Among these, germline defects in *CDH1*, encoding the epithelial cadherin, have been particularly explored in the context of both familial and sporadic gastric cancer development [30]. The worldwide distribution of germline *CDH1* mutations is highly heterogeneous (Fig. 2.1).

2.3 Frequency of E-Cadherin Germline Mutations

In Table 2.1, we reported all *CDH1* germline mutations identified in stomach cancer from 1998 to 2021. The higher frequency of germline mutations was identified in Europe (47.1%), following Asia (22.8%), North America (15.5%), Oceania (7.7%), and South America (6.8%) (Fig. 2.2). Asia reported the higher frequency of missense mutations, in comparison with the other continents (Fig. 2.3).

Recently, we have conducted a systematic study aiming to collect all *CDH1* germline mutations described in the literature and detected in stomach cancer [31].

Table 2.1 List of germline CDH1 mutations identified in the world

First Author	Year	Country	Type	HGVS	Protein change	CDH1posKG
Guilford [1]	1998	Maori	Splice-site	1008G > T	/	9
		Maori	Non-sense	2095C > T	/	1
		Maori	Frameshift	2386dupC	/	2
Gayther [32]	1998	Europe	Non-sense	187C > T	/	2
		Europe	Insertion	1711 + 1dupG	/	3
		Europe	Non-sense	1792C > T	/	2
Guilford [33]	1999	Europe	Insertion	1588insC	/	3
		Europe	Non-sense	70G > T	/	3
		USA	Splice-site	1137 + 1G > A	/	3
		Europe	Non-sense	586G > T	/	1
		Maori	Non-sense	190C > T	/	1
		Unknown	Frameshift	1487del7	/	1
Keller [34]	1999	Germany	Deletion	377delC	/	2
Richards [35]	1999	Ireland	Splice-site	49-2A > G	/	1
		Ireland	Non-sense	59G > A	/	1
Shinmura [36]	1999	Japan	Missense	185G > T	G62V	1
Yoon [37]	1999	Korea	Missense	731A > G	D244G	1
		Korea	Missense	1460 T > C	V487G	1
Kim [38]	2000	Korea	Missense	1018A > G	T340A	6
Dussaulx-Garin [39]	2001	France	Non-sense	283C > T	/	3
Ascano [40]	2001	USA	Missense	1849G > A	A617T	1
Oliveira [41]	2002	Pakistan	Splice-site	832G > A	/	3
		Europe	Missense	1018A > G	T340A	1
		Europe	Insertion	1472dupA	/	1
		Europe	Insertion	45insT	/	1
Yabuta [42]	2002	Japan	Missense	2494G > A	V832M	1

(continued)

Table 2.1 (continued)

First Author	Year	Country	Type	HGVS	Protein change	CDH1posKG
Humar [43]	2002	Caucasian	Deletion	53delC	/	1
		Arabia	Splice-site	1565 + 1G > T	/	1
		Maori	Non-sense	1792C > T	/	1
		Caucasian	Splice-site	2295 + 5G > A	/	1
		USA	Deletion	1710delT	/	2
Suriano [44]	2003	Portugal	Missense	1901C > T	A634V	1
		UK	Splice-site	532-18C > T	/	1
		Portugal	Splice-site	532-18C > T	/	1
Wang [45]	2003	Japan	Missense	1243A > C	I415L	3
Oliveira [46]	2004	Portugal	Del-ins	1135_1137 + 5delinsTTAGA	/	1
		Portugal	Missense	1901C > T	A634V	1
Jiang [47]	2004	China	Non-sense	1507C > T	/	1
Oliveira [48]	2004	Europe	Deletion	1135_1137 + 5delinsTTAGA	/	4
Brooks-Wilson [4]	2004	Europe	Splice-site	687 + 1G > A	/	1
		Caucasian	Insertion	1779insC	/	1
		Unknown	Missense	1226 T > C	W409R	1
		Caucasian	Deletion	1212delC	/	1
		Italy	Deletion	382delC	/	1
		Unknown	Deletion	1476_1477delAGdel	/	1
		Caucasian	Insertion	1064dupT	/	1
		Europe	Missense	892G > A	A298T	1
Keller [49]	2004	Germany	Deletion	372delC	/	1
		Germany	Missense	2396C > G	P799R	1
		Germany	Insertion	1619dupG	/	1
Suriano [50]	2005	Unknown	Missense	3G > C	M1I	1

		Unknown	Deletion	1063del	/	1
		Unknown	Non-sense	187C > T	/	1
		Unknown	Non-sense	1792C > T	/	1
		Hispanic	Splice-site	2161C > G	/	1
		Unknown	Non-sense	1003C > T	/	2
		Unknown	Deletion	2276delG	/	1
		Caucasian	Missense	1285C > T	P429S	1
Rodriguez-Sanjuan [51]	2006	Spain	Deletion	1610delC	/	3
Bacani [52]	2006	Canada	Splice-site	-117G > A	/	1
		Canada	Splice-site	-71C > G	/	2
		Canada	Deletion	41delT	/	1
		Canada	Splice-site	48 + 5C > G	/	1
		Canada	Splice-site	48 + 15C > G	/	2
		Canada	Splice-site	387 + 26C > T	/	1
		Canada	Splice-site	1937-13 T > C	/	2
		Canada	Splice-site	2295 + 53G > A	/	1
Zhang [53]	2006	China	Missense	1018A > G	T340A	2
More [54]	2007	Caucasian	Splice-site	49-2A > C	/	3
		Caucasian	Missense	353C > G	T118R	1
		Caucasian	Missense	715G > A	G239R	1
		Hispanic	Deletion	1107delC	/	1
		Caucasian	Splice-site	1137G > A	/	2
		Caucasian	Deletion	1391_1392delTC	/	1
		Maori	Missense	1901C > T	A634V	1
		China	Non-sense	2095C > T	/	1
		Caucasian	Splice-site	2440-6C > G	/	1
Norton [55]	2007	USA	Non-sense	1003C > T	/	11

(continued)

Table 2.1 (continued)

First Author	Year	Country	Type	HGVS	Protein change	CDH1posKG
Van Domselaar [56]	2007	Spain	Non-sense	1913G > A	/	1
Kaurah [3]	2007	Unknown	Non-sense	283C > T	/	1
		Filipine	Missense	715G > A	G239R	1
		Unknown	Splice-site	1137G > A	/	1
		Italy	Splice-site	1137G > A	/	2
		Sweden	Splice-site	1137G > A	/	1
		UK	Deletion	1397-1398delTC	/	1
		Ireland	Insertion	1682dupA	/	1
		UK	Missense	1901C > T	A634V	2
		Portugal	Missense	1901C > T	A634V	1
		Portugal	Missense	1901C > T	A634V	1
		Spanish	Non-sense	1913G > A	/	1
		Germany	Deletion	2064-2065delTG	/	2
		UK	Deletion	2064-2065delTG	/	1
		Unknown	Splice-site	2164 + 5G > A	/	1
		Unknown	Missense	2195G > A	R732Q	1
		UK	Missense	2195G > A	R732Q	3
		Colombia	Missense	2245C > T	R749W	1
		UK	Missense	2343A > T	E781D	1
		France	Deletion	2398delC	/	2
		Ireland	Deletion	2398delC	/	1
		Ireland	Deletion	2398delC	/	2
		Ireland	Deletion	2398delC	/	4
Roviello [57]	2007	Italy	Missense	1118C > T	P373L	1
Rogers [58]	2008	USA	Insertion	1565 + 2insT	/	3

		USA	Deletion	2395delC	/	1
Caron [59]	2008	France	Deletion	2399delG	/	6
Lynch [60]	2008	USA	Non-sense	1003C > T	/	11
		USA	Non-sense	70G > T	/	5
		USA	Missense	2195G > A	R732Q	3
		USA	Non-sense	1792C > T	/	1
Frebourg [61]	2009	Caucasian	Splice-site	531 + 2 T > A	/	4
Oliveira [62]	2009	North Europa	Deletion	Del exon 1–2	/	1
		Canada	Deletion	Del exon 1–2	/	1
		East Europa	Deletion	Del exon 1–2	/	1
		South Europa	Deletion	Del 50-UTR-exon 1	/	1
		Central Europa	Deletion	Del exon 14–16	/	1
		Central Europa	Deletion	Del exon 16	/	1
Mayrbaeurl [63]	2010	Austria	Del-ins	1304_1305delinsA	/	7
Ghaffari [64]	2010	Iran	Non-sense	2275G > T	/	0
Kluijt [65]	2011	Netherland	Deletion	55_74del20	/	1
		Netherland	Non-sense	187C > T	/	1
		Netherland	Non-sense	489C > A	/	3
		Hindustan	Del-ins	811_812delins12	/	2
		Netherland	Del-ins	1135_1137 + 5delinsTTAGA	/	1
		Netherland	Deletion	1404delC	/	9
		Netherland	Deletion	1476_1477delAG	/	1
		Netherland	Ins-Splice-site	1565 + 2dupT	/	14
		Creole	Missense	1748 T > G	L583R	2
		Turkey	Missense	2195G > A	R732Q	2
Yamada [66]	2011	Japan	Deletion	1212delC	/	1
		Japan	Deletion	164-?_387 +?del	/	1

(continued)

Table 2.1 (continued)

First Author	Year	Country	Type	HGVS	Protein change	CDH1posKG
Corso [67]	2011	Italy	Splice-site	-63C > A	/	1
Shah [68]	2012	USA	Non-sense	1792C > T	/	2
Garziera [69]	2013	Italy	Splice-site	-71C > G	/	3
		Italy	Missense	820G > A	G274S	1
		Italy	Missense	892G > A	A298T	1
		Italy	Missense	1409C > T	T470I	1
		Italy	Splice-site	1937-13 T > C	/	2
		Italy	Missense	1774G > A	A592T	1
Chen [70]	2013	China	Deletion	44_46delTGC	/	2
		China	Missense	604G > A	V202I	1
		China	Missense	1888C > G	L630V	1
		China	Splice-site	1320 + 7A > G	/	1
Benusiglio [71]	2013	France	Non-sense	1147C > T	/	1
		France	Deletion	Del exon 11	/	1
		France	Splice-site	832 + 1G > T	/	2
		France	Deletion	2398delC	/	1
		France	Insertion	1565 + 2dup	/	1
		France	Splice-site	1137G > A	/	1
		France	Deletion	1470–1483del	/	1
		France	Splice-site	1679C > G	/	1
		France	Deletion	469delG	/	1
		France	Non-sense	283C > T	/	1
		France	Non-sense	1595G > A	/	1
Kim [72]	2013	Korea	Missense	715G > A	G239R	1
		Korea	Non-sense	1003C > T	/	1

		Korea	Non-sense	1003C > T	/	1
Tsukanov [73]	2013	Russia	Deletion	1005delA	/	1
Zhang [74]	2014	Hispanic	Missense	48G > C	Q16H	2
Sugimoto [75]	2014	Japan	Deletion	1566-?_1711 +?del	/	1
Yamada [76]	2014	Japan	Deletion	Large del exons 7–16	/	2
Bardram [77]	2014	Denmark	Deletion	602_603delCT	/	1
		Denmark	Insertion	1565 + 3insTT	/	5
Black [78]	2014	Hawaii	Non-sense	Trp20stop	/	1
Molinaro [79]	2014	Italy	Splice-site	688-1G > C	/	1
		Italy	Missense	2315 T > A	L772Q	1
		Italy	Deletion	833-476_1138-463del	/	1
		Italy	Imbalance	Allelic imbalance	/	1
		Italy	Non-sense	187C > T	/	1
		Italy	Missense	1901C > T	A634V	1
Hansford [7]	2015	Mexico	Deletion	del 67328695-67328844	/	1
		Lithuania	Deletion	del 67324886-67330557	/	1
		East Europe	Deletion	1-?_163+?del	/	1
		Ireland	Missense	3G > A	M1I	2
		Canada	Splice-site	48 + 1G > A	/	1
		Hispanic	Missense	286A > G	I96V	1
		Lebanon	Deletion	382delC	/	1
		UK	Deletion	447_453delCAGAAGA	/	1
		Algeria	Splice-site	687 + 1G > T	/	1
		Hispanic	Missense	715G > A	G239R	1
		Germany	Splice-site	832 + 1G > T	/	1
		Caucasian	Splice-site	833-2A > G	/	1
		Europe	Missense	892G > A	A298T	1

(continued)

Table 2.1 (continued)

First Author	Year	Country	Type	HGVS	Protein change	CDH1posKG
		Mexico	Non-sense	940A > T	/	1
		Unknown	Splice-site	1137 + 1G > A	/	1
		Unknown	Insertion	1565 + 2dupT	/	1
		Caucasian	Missense	1679C > G	T560R	1
		Irish	Non-sense	1792C > T	/	1
		Hispanic	Non-sense	1914G > A	/	1
		Brazil	Deletion	2058_2059delTG	/	1
		UK	Deletion	2100delT	/	1
		Unknown	Deletion	2310delC	/	1
		UK	Deletion	2430delT	/	1
Yelskaya [80]	2016	USA	Splice-site	1679C > G	/	3
López [81]	2016	Spain	Deletion	1220delC	/	3
El-Husny [82]	2016	Brazil	Nonsense	1023T > G	/	2
Betés [83]	2017	Spain	Missense	977T > A	I326N	3
Kim [84]	2017	Korea	Missense	2494G > A	V832M	2
Pena-Couso [85]	2018	Spain	Missense	1679C > G	T560R	3
		Spain	Missense	1679C > G	T560R	2
		Spain	Missense	1679C > G	T560R	2
Gullo [86]	2018	Portugal	Missense	1901C > T	A634V	7
Caggiari [87]	2018	Italy	Deletion	1612delG	/	1
Norero [88]	2019	Chile	Non-sense	1531C > T	/	2
Katona [89]	2019	USA	Splice-site	1566-2A > G	/	1
Obermair [90]	2019	Austria	Splice-site	687 + 1G > A	/	3
Guindalini [91]	2019	Brazil	Missense	313 T > A	S105T	1
		Brazil	Missense	387G > T	Q129H	1

		Brazil	Missense	1676G > A	S559N	1
		Brazil	Missense	1806C > A	F602L	1
Hakkaart [92]	2019	New Zealand	Non-sense	190C > T	/	4
		New Zealand	Non-sense	1792C > T	/	4
		New Zealand	Non-sense	2195G > A	/	1
		New Zealand	Non-sense	2287G > T	/	4
		New Zealand	Insertion	2382insC	/	4
Tedaldi [93]	2019	Italy	Deletion	1-?_163+?del	/	1
		Italy	Non-sense	308G > A	/	1
		Italy	Deletion	360delG	/	1
		Italy	Non-sense	781G > T	/	2
		Italy	Non-sense	1003C > T	/	1
		Italy	Non-sense	1137G > A	/	1
		Italy	Deletion	1965delG	/	1
		Italy	Deletion	2114delT	/	1
Shin [94]	2020	Korea	Missense	2494G > A	V832M	7
Zhang [95]	2021	China	Deletion	1475_1479del	/	1
Ithurralde-Argerich [96]	2021	Argentina	Non-sense	1531C > T	511G	8
Dang [97]	2021	Vietnam	Non-sense	639G > A	W213	1
Hirakawa [98]	2021	Japan	Missense	1679C > G	T560R	3
Valenzuela [99]	2021	Mexico	Deletion	377del	/	1
Pan [100]	2021	China	Missense	1018A > G	T340A	3
		China	Non-sense	489C > A	/	1
		China	Missense	2638G > A	E880K	1
		China	Missense	1888C > G	L630V	3
		China	Missense	1118C > T	P373L	1
		China	Non-sense	1225G > A	/	1

(continued)

Table 2.1 (continued)

First Author	Year	Country	Type	HGVS	Protein change	CDH1posKG
		China	Missense	1651G > C	E551Q	6
		China	Missense	1296C > G	N432K	1
		China	Non-sense	187C > T	/	1
		China	Missense	1213A > T	N405Y	1
		China	Missense	1019C > T	T340M	1
Namikawa [101]	2021	Japan	Deletion	603del	/	1
Vidal [102]	2021	Brazil	Non-sense	1023 T > G	T341	1

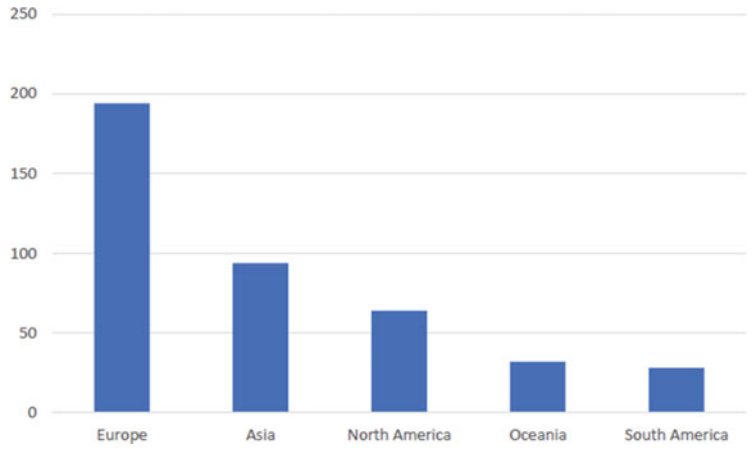


Fig. 2.2 Geographic distribution of germline *CDH1* mutations per continent

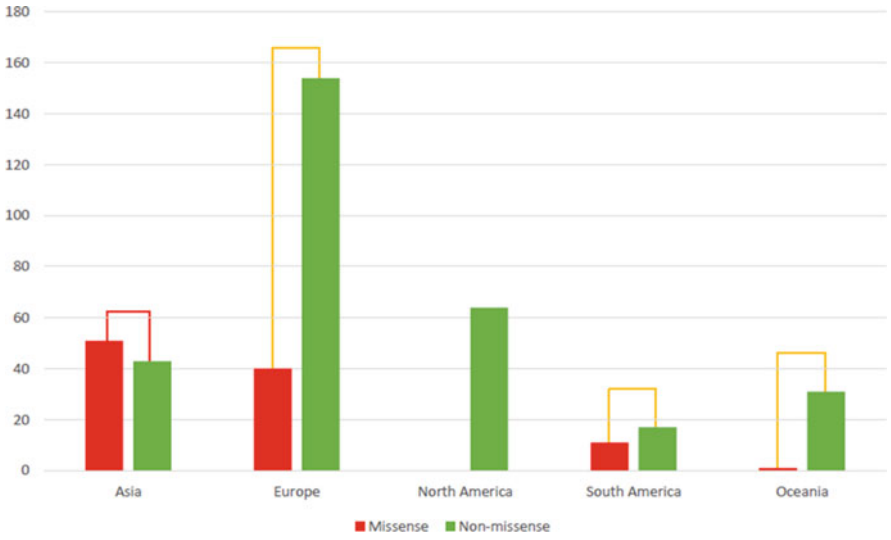


Fig. 2.3 Frequency of germline *CDH1* missense mutations in the world

Given that prevalence of GC is not homogenous worldwide, the geographical distribution of mutations was classified above as "series and family studies" groups. In the series study group, it has been verified that 45.5% of *CDH1* alterations were detected in individuals from European origin, with lower percentages identified in Asian and American individuals (26.3 and 16.0%, respectively), as well as in Oceania (11.5%). Remarkably, it has been verified that the predominant mutation type varies across geographical regions. Deletions are more frequent in Europe (34%), splice-site in America (48%), missense in Asia (68%), and non-sense in

Oceania (78%). The high prevalence of missense mutations in Asia is mainly attributed to Korean and Japanese populations. Despite obvious differences in the number of mutations reported in each setting (series or family study), it has been demonstrated that, in the family study context, 51.9% of mutations are of European origin, 26.6% of American, 15.6% of Oceania, and 5.9% of Asian origins. In this group, a distinct distribution pattern of mutation classes was also observed worldwide. Deletions (33%) and missense (33%) alterations are the most common alteration type in Europe, non-sense in America (69%), deletions in Asia (47%), and splice-site in Oceania (87%). A striking difference was found in Europe, where the relative frequency of splice-site alterations decreases from series to family studies, while the missense category increases. Likewise, in the American continent, splice-site alterations decrease from 48% in the series study group to 10% in the family group, which in turn depicts a substantial increase of non-sense mutations. The opposite effect is verified in Oceania: splice-site alterations, that were not identified in the series study, appear in 87% of the family study subjects; whereas the non-sense relative frequency decreases from 78% to 4%. Another difference is detected at the Asian region and involves missense variants, where a very high frequency of these variants (78%) occurs in the series study but not in the family study group. This could be a result of low penetrance of missense variants, in turn leading to their underestimation and, consequently, segregation within populations. We observe that, along with other host genetic and environmental factors, *CDH1* missense variants are associated with the high incidence of gastric cancer in Asian populations. *CDH1* missense mutations occur as sparse events in countries with low-incidence for GC, such as the USA, New Zealand, France, Canada, and the UK, but are frequent in Korea, Japan, China, and Italy, which are high-incidence countries (9% vs. 51% respectively) (Fig. 2.2) [29].

2.4 Conclusion

CDH1 mutations are more frequently identified in countries with low incidence for GC, the application of criteria for genetic screening is critical for a higher detection rate, as well as for the identification of mutations with proven clinical relevance. Recent results from systematic analysis clearly have corroborated that following guidelines for screening, and surveillance of patients at high risk has the potential to diagnose and treat GC at an earlier stage, improving survival rates. In the absence of clinical criteria and of familial genotype-phenotype correlation, detection of a *CDH1* mutation imposes a clinical management issue given that the consensual risk-reducing recommendation regarding DGC is a radical and life-changing gastrectomy. Those individuals should be closely monitored through an intense surveillance program, which should both contribute to early diagnosis and to enlighten disease etiology.

Note

Parts of this chapter are based on the open access publication by Corso G. et al. 2021 [103].

References

1. Guilford P, Hopkins J, Harraway J et al (1998) E-cadherin germline mutations in familial gastric cancer. Nature 392(6674):402–405. https://doi.org/10.1038/32918
2. Caldas C, Carneiro F, Lynch HT et al (1999) Familial gastric cancer: overview and guidelines for management. J Med Genet 36(12):873–880. https://doi.org/10.1136/jmg.36.12.873
3. Kaurah P, MacMillan A, Boyd N et al (2007) Founder and recurrent CDH1 mutations in families with hereditary diffuse gastric cancer. JAMA 297(21):2360–2372. https://doi.org/10.1001/jama.297.21.2360
4. Brooks-Wilson AR, Kaurah P, Suriano G et al (2004) Germline E-cadherin mutations in hereditary diffuse gastric cancer: assessment of 42 new families and review of genetic screening criteria. J Med Genet 41(7):508–517. https://doi.org/10.1136/jmg.2004.018275
5. Van der Pos RS, Vogelaar IP, Carneiro F et al (2015) Hereditary diffuse gastric cancer: Updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. J Med Genet 52(6):361–374. https://doi.org/10.1136/jmedgenet-2015-103094
6. Corso G, Figueiredo J, La Vecchia C et al (2018) Hereditary lobular breast cancer with an emphasis on E-cadherin genetic defect. J Med Genet 55(7):431–441. https://doi.org/10.1136/jmedgenet-2018-105337
7. Hansford S, Kaurah P, Li-Chang H et al (2015) Hereditary diffuse gastric cancer syndrome: CDH1 mutations and beyond. JAMA Oncol 1(1):23–32. https://doi.org/10.1001/jamaoncol.2014.168
8. Suriano G, Seixas S, Rocha J et al (2006) A model to infer the pathogenic significance of CDH1 germline missense variants. J Mol Med 84(12):1023–1031. https://doi.org/10.1007/s00109-006-0091-z
9. Lee K, Krempely K, Roberts ME et al (2018) Specifications of the ACMG/AMP variant curation guidelines for the analysis of germline CDH1 sequence variants. Hum Mutat 39(11):1553–1568. https://doi.org/10.1002/humu.23650
10. Chakravorty S, Hegde M (2017) Gene and variant annotation for mendelian disorders in the era of advanced sequencing technologies. Ann Rev Genom Hum Genet 31(18):229–256. https://doi.org/10.1146/annurev-genom-083115-022545
11. Corso G, Marrelli D, Pascale V et al (2012) Frequency of CDH1 germline mutations in gastric carcinoma coming from high- and low-risk areas: metanalysis and systematic review of the literature. BMC Cancer. https://doi.org/10.1186/1471-2407-12-8
12. Lyons K, Le LC, Pham YT et al (2019) Gastric cancer: epidemiology, biology, and prevention: a mini review. Eur J Cancer Prev 28(5):397–412. https://doi.org/10.1097/CEJ.0000000000000480
13. GBD 2017 Stomach Cancer Collaborators (2020) The global, regional, and national burden of stomach cancer in 195 countries, 1990–2017: a systematic analysis for the global burden of disease study 2017. Lancet Gastroenterol Hepatol 5(1):42–54. https://doi.org/10.1016/S2468-1253(19)30328-0
14. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Abate D et al (2019) Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2017: a systematic analysis for the global burden of disease study. JAMA Oncol 5(12):1749–1768. https://doi.org/10.1001/jamaoncol.2019.2996

15. Ladeiras-Lopes R, Pereira AK, Nogueira A et al (2008) Smoking and gastric cancer: systematic review and meta-analysis of cohort studies. Cancer Causes Control 19(7):689–701. https://doi.org/10.1007/s10552-008-9132-y
16. Siegel R, Ma J, Zou Z et al (2014) Cancer statistics. CA Cancer J Clin 64(1):9–29. https://doi.org/10.3322/caac.21208
17. Matsuda T, Ajiki W, Marugame T et al (2011) Population-based survival of cancer patients diagnosed between 1993 and 1999 in Japan: a chronological and international comparative study. Jpn J Clin Oncol 41(1):40–51. https://doi.org/10.1093/jjco/hyq167
18. Oh CM, Won YJ, Jung KW et al (2016) Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2013. Cancer Res Treat 48(2):436–450. https://doi.org/10.4143/crt.2016.089
19. Carioli G, Bertuccio P, Boffetta P et al (2020) European cancer mortality predictions for the year 2020 with a focus on prostate cancer. Ann Oncol 31(5):650–668. https://doi.org/10.1016/j.annonc.2020.02.009
20. Saghier AA, Sagar M, Kabanja JH et al (2013) Gastric cancer: environmental risk factors, treatment and prevention. J Carcinogene Mutagene S14:008. https://doi.org/10.4172/2157-2518.S14-008
21. Ward E, Jemal A, Forman D et al (2011) Global cancer statistics. CA Cancer J Clin 61(2):69–90. https://doi.org/10.3322/caac.20107
22. Nagini S (2012) Carcinoma of the stomach: a review of epidemiology, pathogenesis, molecular genetics and chemoprevention. World J Gastrointest Oncol 4(7):156–169. https://doi.org/10.4251/wjgo.v4.i7.156
23. Piazuelo MB, Correa P (2013) Gastric cancer: overview. Colomb Med 44(3):192–201. https://doi.org/10.25100/cm.v44i3.1263
24. Karimi P, Islami F, Anandasabapathy S et al (2014) Gastric cancer: descriptive epidemiology, risk factors, screening, and prevention. Cancer Epidemiol Biomark Prev 23(5):700–713. https://doi.org/10.1158/1055-9965.EPI-13-1057
25. Ferlay J, Parkin DM, Steliarova-Foucher E (2010) Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer 46(4):765–781. https://doi.org/10.1016/j.ejca.2009.12.014
26. Ferlay J, Shin HR, Bray F et al (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 127(12):2893–2917. https://doi.org/10.1002/ijc.25516
27. Ferro A, Peleteiro B, Malvezzi M et al (2014) Worldwide trends in gastric cancer mortality (1980–2011), with predictions to 2015, and incidence by subtype. Eur J Cancer 50(7):1330–1344. https://doi.org/10.1016/j.ejca.2014.01.029
28. La Vecchia C, Negri E, Franceschi S et al (1992) A family history and the risk of stomach and colorectal cancer. Cancer 70(1):50–55. https://doi.org/10.1002/1097-0142(19920701)70:1<50::aid-cncr2820700109>3.0.co;2-i
29. Corso G, Montagna G, Figueiredo J et al (2020) Hereditary gastric and breast cancer syndromes related to CDH1 germline mutation: a multidisciplinary clinical review. Cancers 12(6):1598. https://doi.org/10.3390/cancers12061598
30. Corso G, Carvalho J, Marrelli D et al (2013) Somatic mutations and deletions of the E-cadherin gene predict poor survival of patients with gastric cancer. J Clin Oncol 31(7):868–875. https://doi.org/10.1200/JCO.2012.44.4612
31. Corso G, Bonanni B (2020) E-cadherin (CDH1 gene) germline mutations in gastric cancer: evolutions and innovations. Cancers 12(10):2920. https://doi.org/10.3390/cancers12102920
32. Gayther SA, Gorringe KL, Ramus SJ et al (1998) Identification of germ-line E-cadherin mutations in gastric cancer families of European origin. Cancer Res 58(18):4086–4089
33. Guilford PJ, Hopkins JB, Grady WM et al (1999) E-cadherin germline mutations define an inherited cancer syndrome dominated by diffuse gastric cancer. Hum Mutat 14(3):249–255. https://doi.org/10.1002/(SICI)1098-1004(1999)14:3<249::AID-HUMU8>3.0.CO;2-9
34. Keller G, Vogelsang H, Becker I et al (1999) Diffuse type gastric and lobular breast carcinoma in a familial gastric cancer patient with an E-cadherin germline mutation. Am J Pathol 155(2):337–342. https://doi.org/10.1016/S0002-9440(10)65129-2

35. Richards FM, McKee SA, Rajpar MH et al (1999) Germline E-cadherin gene (CDH1) mutations predispose to familial gastric cancer and colorectal cancer. Hum Mol Genet 8(4): 607–610. https://doi.org/10.1093/hmg/8.4.607
36. Shinmura K, Kohno T, Takahashi M et al (1999) Familial gastric cancer: clinicopathological characteristics, RER phenotype and germline p53 and E-cadherin mutations. Carcinogenesis 20(6):1127–1131. https://doi.org/10.1093/carcin/20.6.1127
37. Yoon KA, Ku JL, Yang HK et al (1999) Germline mutations of E-cadherin gene in Korean familial gastric cancer patients. J Hum Genet 44(3):177–180. https://doi.org/10.1007/s100380050137
38. Kim HC, Wheeler JM, Kim JC et al (2000) (2000) The E-cadherin gene (CDH1) variants T340A and L599V in gastric and colorectal cancer patients in Korea. Gut 47(2):262–267. https://doi.org/10.1136/gut.47.2.262
39. Dussaulx-Garin L, Blayau M, Pagenault M et al (2001) A new mutation of E-cadherin gene in familial gastric linitis plastica cancer with extra-digestive dissemination. Eur J Gastroenterol Hepatol 13(6):711–715. https://doi.org/10.1097/00042737-200106000-00016
40. Ascaño JJ, Frierson H Jr, Moskaluk CA et al (2001) Inactivation of the E-cadherin gene in sporadic diffuse-type gastric cancer. Mod Pathol 14(10):942–949. https://doi.org/10.1038/modpathol.3880416
41. Oliveira C, Bordin MC, Grehan N et al (2002) Screening E-cadherin in gastric cancer families reveals germline mutations only in hereditary diffuse gastric cancer kindred. Hum Mutat 19(5):510–517. https://doi.org/10.1002/humu.10068
42. Yabuta T, Shinmura K, Tani M et al (2002) E-cadherin gene variants in gastric cancer families whose probands are diagnosed with diffuse gastric cancer. Int J Cancer 101(5):434–441. https://doi.org/10.1002/ijc.10633
43. Humar B, Toro T, Graziano F et al (2002) Novel germline CDH1 mutations in hereditary diffuse gastric cancer families. Hum Mutat 19(5):518–525. https://doi.org/10.1002/humu.10067
44. Suriano G, Oliveira C, Ferreira P et al (2003) Identification of CDH1 germline missense mutations associated with functional inactivation of the E-cadherin protein in young gastric cancer probands. Hum Mol Genet 12(5):575–582. https://doi.org/10.1093/hmg/ddg048
45. Wang Y, Song JP, Ikeda M et al (2003) Ile-Leu substitution (I415L) in germline E-cadherin gene (CDH1) in Japanese familial gastric cancer. Jpn J Clin Oncol 33(1):17–20. https://doi.org/10.1093/jjco/hyg002
46. Oliveira C, Suriano G, Ferreira P et al (2004) Genetic screening for familial gastric cancer. Hered Cancer Clin Pract 2(2):51–64. https://doi.org/10.1002/humu.10068
47. Jiang Y, Wan YL, Wang ZJ et al (2004) Germline E-cadherin gene mutation screening in familial gastric cancer kindreds. Zhonghua Wai Ke Za Zhi 42(15):914–917
48. Oliveira C, de Bruin J, Nabais S et al (2004) Intragenic deletion of CDH1 as the inactivating mechanism of the wild-type allele in a HDGC tumour. Oncogene 23(12):2236–2240. https://doi.org/10.1038/sj.onc.1207335
49. Keller G, Vogelsang H, Becker I et al (2004) (2004) Germline mutations of the E-cadherin (CDH1) and TP53 genes, rather than of RUNX3 and HPP1, contribute to genetic predisposition in German gastric cancer patients. J Med Genet 41(6):e89. https://doi.org/10.1136/jmg.2003.015594
50. Suriano G, Yew S, Ferreira P et al (2005) Characterization of a recurrent germ line mutation of the E-cadherin gene: implications for genetic testing and clinical management. Clin Cancer Res. 11(15):5401–5409. https://doi.org/10.1158/1078-0432.CCR-05-0247
51. Rodriguez-Sanjuan JC, Fontalba A, Mayorga M et al (2006) A novel mutation in the E-cadherin gene in the first family with hereditary diffuse gastric cancer reported in Spain. Eur J Surg Oncol. 32(10):1110–1113. https://doi.org/10.1016/j.ejso.2006.06.006
52. Bacani JT, Soares M, Zwingerman R et al (2006) CDH1/E-cadherin germline mutations in early-onset gastric cancer. J Med Genet 43(11):867–872. https://doi.org/10.1136/jmg.2006.043133

53. Zhang Y, Liu X, Fan Y et al (2006) Germline mutations and polymorphic variants in MMR, E-cadherin and MYH genes associated with familial gastric cancer in Jiangsu of China. Int J Cancer 119(11):2592–2596. https://doi.org/10.1002/ijc.22206
54. More H, Humar B, Weber W et al (2007) Identification of seven novel germline mutations in the human E-cadherin (CDH1) gene. Hum Mutat. 28(2):203. https://doi.org/10.1002/humu.9473
55. Norton JA, Ham CM, Van Dam J et al (2007) CDH1 truncating mutations in the E-cadherin gene: an indication for total gastrectomy to treat hereditary diffuse gastric cancer. Ann Surg. 245(6):873–879. https://doi.org/10.1097/01.sla.0000254370.29893.e4
56. Van Domselaar F, Correa D, Vaccaro C et al (2007) Hereditary diffuse gastric cancer (HDGC): presentation of a family with a new mutation of the CDH1 gene. Acta Gastroenterol Latinoam. 37(3):158–163
57. Roviello F, Corso G, Pedrazzani C et al (2007) Hereditary diffuse gastric cancer and E-cadherin: description of the first germline mutation in an Italian family. Eur J Surg Oncol 33(4):448–451. https://doi.org/10.1016/j.ejso.2006.10.028
58. Rogers WM, Dobo E, Norton JA et al (2008) Risk-reducing total gastrectomy for germline mutations in E-cadherin (CDH1): pathologic findings with clinical implications. Am J Surg Pathol. 32(6):799–809. https://doi.org/10.1097/PAS.0b013e31815e7f1a
59. Caron O, Schielke A, Svrcek M et al (2008) Usefulness of prophylactic gastrectomy in a novel large hereditary diffuse gastric cancer (HDGC) family. Am J Gastroenterol 103(8):2160–2161. https://doi.org/10.1111/j.1572-0241.2008.0198217.x
60. Lynch HT, Kaurah P, Wirtzfeld D et al (2008) Hereditary diffuse gastric cancer: diagnosis, genetic counseling, and prophylactic total gastrectomy. Cancer 112(12):2655–2663. https://doi.org/10.1002/cncr.23501
61. Frebourg T, Laurent-Puig P, Benamouzig R et al (2009) Genomics and genetics genome projects. Med Sci (Paris) 25(1):33–37. https://doi.org/10.1051/medsci/2009251s33
62. Oliveira C, Senz J, Kaurah P et al (2009) Germline CDH1 deletions in hereditary diffuse gastric cancer families. Hum Mol Genet 18(9):1545–1555. https://doi.org/10.1093/hmg/ddp046
63. Mayrbaeurl B, Keller G, Schauer W et al (2010) Germline mutation of the E-cadherin gene in three sibling cases with advanced gastric cancer: clinical consequences for the other family members. Eur J Gastroenterol Hepatol 22(3):306–310. https://doi.org/10.1097/MEG.0b013e32832bab9a
64. Ghaffari SR, Rafati M, Sabokbar T et al (2010) A novel truncating mutation in the E-cadherin gene in the first Iranian family with hereditary diffuse gastric cancer. Eur J Surg Oncol 36(6):559–562. https://doi.org/10.1016/j.ejso.2010.04.001
65. Kluijt I, Siemerink EJ, Ausems MG et al (2012) NCDH1-related hereditary diffuse gastric cancer syndrome: clinical variations and implications for counseling. Int J Cancer. 131(2):367–376. https://doi.org/10.1002/ijc.26398
66. Yamada H, Shinmura K, Ito H et al (2011) Germline alterations in the CDH1 gene in familial gastric cancer in the Japanese population. Cancer Sci 102(10):1782–1788. https://doi.org/10.1111/j.1349-7006.2011.02038.x
67. Corso G, Pedrazzani C, Pinheiro H et al (2011) (2011) E-cadherin genetic screening and clinico-pathologic characteristics of early onset gastric cancer. Eur J Cancer. 47(4):631–639. https://doi.org/10.1016/j.ejca.2010.10.011
68. Shah MA, Salo-Mullen E, Stadler Z et al (2012) De novo CDH1 mutation in a family presenting with early-onset diffuse gastric cancer. Clin Genet 82(3):283–287. https://doi.org/10.1111/j.1399-0004.2011.01744.x
69. Garziera M, Canzonieri V, Cannizzaro R et al (2013) Identification and characterization of CDH1 germline variants in sporadic gastric cancer patients and in individuals at risk of gastric cancer. PLoS One 8(10):e77035. https://doi.org/10.1371/journal.pone.0077035

70. Chen QH, Deng W, Li XW et al (2013) Novel CDH1 germline mutations identified in Chinese gastric cancer patients. World J Gastroenterol. 19(6):909–916. https://doi.org/10.3748/wjg.v19.i6.909
71. Benusiglio PR, Malka D, Rouleau E et al (2013) CDH1 germline mutations and the hereditary diffuse gastric and lobular breast cancer syndrome: a multicentre study. J Med Genet. 50(7):486–489. https://doi.org/10.1136/jmedgenet-2012-101472
72. Kim S, Chung JW, Jeong TD et al (2013) Searching for E-cadherin gene mutations in early onset diffuse gastric cancer and hereditary diffuse gastric cancer in Korean patients. Fam Cancer. 12(3):503–507. https://doi.org/10.1007/s10689-012-9595-6
73. Tsukanov AC, Shelygin IA, Kashnikov VN et al (2013) Molecular genetics study of hereditary predisposition to diffuse gastric cancer in Russian patients. Vopr Onkol. 59(5):580–584
74. Zhang L, Xiao A, Ruggeri J et al (2014) The germline CDH1 c.48 G>C substitution contributes to cancer predisposition through generation of a pro-invasive mutation. Mutat Res. 770:106–111. https://doi.org/10.1016/j.mrfmmm.2014.10.001
75. Sugimoto S, Yamada H, Takahashi M et al (2014) Early-onset diffuse gastric cancer associated with a de novo large genomic deletion of CDH1 gene. Gastric Cancer 17(4):745–749. https://doi.org/10.1007/s10120-013-0278-2
76. Yamada M, Fukagawa T, Nakajima T et al (2014) Hereditary diffuse gastric cancer in a Japanese family with a large deletion involving CDH1. Gastric Cancer 17(4):750–756. https://doi.org/10.1007/s10120-013-0298-y
77. Bardram L, Hansen TV, Gerdes AM et al (2014) Prophylactic total gastrectomy in hereditary diffuse gastric cancer: identification of two novel CDH1 gene mutations-a clinical observational study. Fam Cancer 13(2):231–242. https://doi.org/10.1007/s10689-013-9698-8
78. Black MD, Kaneshiro R, Lai JI et al (2014) Hereditary diffuse gastric cancer associated with E-cadherin germline mutation: a case report. Hawaii J Med Public Health 73(7):204–207
79. Molinaro V, Pensotti V, Marabelli M et al (2014) Complementary molecular approaches reveal heterogeneous CDH1 germline defects in Italian patients with hereditary diffuse gastric cancer (HDGC) syndrome. Genes Chromosomes Cancer 53(5):432–445. https://doi.org/10.1002/gcc.22155
80. Yelskaya Z, Bacares R, Salo-Mullen E et al (2016) CDH1 missense variant c.1679C>G (p.T560R) completely disrupts normal splicing through creation of a novel 5' splice site. PLoS One 11(11):e0165654. https://doi.org/10.1371/journal.pone.0165654
81. López M, Cervera-Acedo C, Santibáñez P et al (2016) A novel mutation in the CDH1 gene in a Spanish family with hereditary diffuse gastric cancer. Springerplus 5(1):1181. https://doi.org/10.1186/s40064-016-2852-7
82. El-Husny A, Raiol-Moraes M, Amador M et al (2016) CDH1 mutations in gastric cancer patients from northern Brazil identified by next- generation sequencing (NGS). Genet Mol Biol 39(2):189–198. https://doi.org/10.1590/1678-4685-GMB-2014-0342
83. Betés M, Alonso-Sierra M, Valentí V et al (2017) A multidisciplinary approach allows identification of a new pathogenic CDH1 germline missense mutation in a hereditary diffuse gastric cancer family. Dig Liver Dis 49(7):825–826. https://doi.org/10.1016/j.dld.2017.04.006
84. Kim Y, Cho MY, Kim J et al (2017) Profiling cancer-associated genetic alterations and molecular classification of cancer in Korean gastric cancer patients. Oncotarget 8(41):69888–69905. https://doi.org/10.18632/oncotarget.19435
85. Pena-Couso L, Perea J, Melo S et al (2018) Clinical and functional characterization of the CDH1 germline variant c.1679C>G in three unrelated families with hereditary diffuse gastric cancer. Eur J Hum Genet 26(9):1348–1353. https://doi.org/10.1038/s41431-018-0173-8
86. Gullo I, Devezas V, Baptista M et al (2018) Phenotypic heterogeneity of hereditary diffuse gastric cancer: report of a family with early-onset disease. Gastrointest Endosc 87(6):1566–1575. https://doi.org/10.1016/j.gie.2018.02.008
87. Caggiari L, Miolo G, Canzonieri V et al (2017) A new mutation of the CDH1 gene in a patient with an aggressive signet-ring cell carcinoma of the stomach. Cancer Biol Ther 19(4):254–259. https://doi.org/10.1080/15384047.2017.1394540

88. Norero E, Alarcon MA, Hakkaart C et al (2019) Identification of c.1531C>T pathogenic variant in the CDH1 gene as a novel germline mutation of hereditary diffuse gastric cancer. Int J Mol Sci. 20(20):4980. https://doi.org/10.3390/ijms20204980
89. Katona B, Farengo Clark D, Domchek SM (2020) CDH1 on multigene panel testing: look before you leap. J Natl Cancer Inst 112(4):330–334. https://doi.org/10.1093/jnci/djz229
90. Obermair F, Rammer M, Burghofer J et al (2019) Cleft lip/palate and hereditary diffuse gastric cancer: report of a family harboring a CDH1 c.687 + 1G > A germline mutation and review of the literature. Fam Cancer 18(2):253–260. https://doi.org/10.1007/s10689-018-0111-5
91. Guindalini RSC, Cormedi MCV, Maistro S et al (2019) Frequency of CDH1 germline variants and contribution of dietary habits in early age onset gastric cancer patients in Brazil. Gastric Cancer 22(5):920–931. https://doi.org/10.1007/s10120-019-00945-9
92. Hakkaart C, Ellison-Loschmann L, Day R et al (2019) Germline CDH1 mutations are a significant contributor to the high frequency of early-onset diffuse gastric cancer cases in New Zealand Māori. Fam Cancer 18(1):83–90. https://doi.org/10.1007/s10689-018-0080-8
93. Tedaldi G, Pirini F, Tebaldi M et al (2019) Multigene panel testing increases the number of loci associated with gastric cancer predisposition. Cancers (Basel) 11(9):1340. https://doi.org/10.3390/cancers11091340
94. Shin S, Kim Y, Lee JK et al (2020) Frequency and clinical characteristics of unselected Korean gastric cancer patients with a germline CDH1 V832M mutation. J Cancer 11(1):208–212. https://doi.org/10.7150/jca.36513
95. Zhang YJ, Yang Y, Wei Q et al (2021) A multicenter study assessing the prevalence of germline genetic alterations in Chinese gastric-cancer patients. Gastroenterol Rep (Oxf) 9(4):339–349. https://doi.org/10.1093/gastro/goab020
96. Ithurralde-Argerich J, Rosner L, Rizzolo M et al (2021) Laparoscopic prophylactic total gastrectomy for hereditary diffuse gastric cancer in CDH1 mutation carriers. J Laparoendosc Adv Surg Tech 31(7):729–737. https://doi.org/10.1089/lap.2021.0239
97. Dang DNT, Nguyen HTT, Ngo HD et al (2021) A novel stop codon mutation in exon 5 (c.639G>A) of the cadherin-1 gene in a Vietnamese man with hereditary diffuse gastric cancer: a case report. J Med Case Rep 15(1):263. https://doi.org/10.1186/s13256-021-02837-y
98. Hirakawa M, Takada K, Sato M et al (2020) Case series of three patients with hereditary diffuse gastric cancer in a single family: three case reports and review of literature. World J Gastroenterol. 26(42):6689–6697. https://doi.org/10.3748/wjg.v26.i42.6689
99. Valenzuela C, Castelán-Maldonado E, Carvajal-Zarrabal O et al (2021) First report of a Mexican family with mutation in the CDH1 gene. Mol Genet Genomic Med 8(11):e1208. https://doi.org/10.1002/mgg3.1208
100. Pan Z, Fu Z, Luo C et al (2021) CDH1 germline mutations in a Chinese cohort with hereditary diffuse gastric cancer. J Cancer Res Clin Oncol. https://doi.org/10.1007/s00432-021-03775-4
101. Namikawa K, Kawachi H, Tsugeno Y et al (2021) Detection of multiple intramucosal signet-ring cell carcinomas by white-light endoscopy and magnifying endoscopy with narrow-band imaging in a hereditary diffuse gastric cancer patient with a CDH1 germline mutation. VideoGIE 6(4):163–166. https://doi.org/10.1016/j.vgie.2020.11.020
102. Vidal AF, Sousa Ferraz R, El-Husny A et al (2021) Comprehensive analysis of germline mutations in northern Brazil: a panel of 16 genes for hereditary cancer-predisposing syndrome investigation. BMC Cancer 21(1):363. https://doi.org/10.1186/s12885-021-08089-9
103. Corso G, Corso F, Bellerba F et al (2021) Geographical distribution of E-cadherin germline mutations in the context of diffuse gastric cancer: a systematic review. Cancers (Basel) 13(6):1269. https://doi.org/10.3390/cancers13061269

Hereditary Lobular Breast Cancer: A Newly Defined Syndrome

3

Vanessa Blair, Antonia Girardi, and Giovanni Corso

Abstract

Germline *CDH1* mutations were first identified in 1998 in families with a strong history of early-onset gastric cancer. Now, 25 years on, it appears that *CDH1* pathogenic variants are associated with a significantly increased risk for only two cancer types, diffuse gastric cancer and lobular breast cancer. Only the *diffuse* type of gastric cancer is associated with germline *CDH1* variants, hence the syndrome was named hereditary diffuse gastric cancer. Lobular breast cancer most commonly appears in the context of classical hereditary diffuse gastric cancer syndrome, segregating with gastric cancer in the family. However, in recent years pathogenic *CDH1* variants have been identified in individuals or families with lobular breast cancer *without* any gastric cancer in the family, leading to a new entity being defined: 'hereditary lobular breast cancer'. Recent studies, as well as the International Gastric Cancer Linkage Consortium, remark on the importance of distinguishing between these two syndromes because it is likely the penetrance risks differ and this has clinical implications.

V. Blair (✉)
Department of Surgery, St Marks Breast Centre, University of Auckland, Auckland, New Zealand
e-mail: vanessa@vinerylane.surgery

A. Girardi
Division of Breast Surgery, European Institute of Oncology, IRCCS, Milan, Italy

G. Corso
Division of Breast Surgery, European Institute of Oncology, IRCCS, Milan, Italy

Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_3

3.1 Introduction

The term 'hereditary lobular breast cancer' (HLBC) has only recently appeared in the breast cancer lexicon [1]. The 2020 consensus guidelines on the diagnosis and management of hereditary diffuse gastric cancer (HDGC) defined HLBC, and incorporated it into a comprehensive pathway of care for *CDH1* pathogenic variant carriers [2]. The definition of HLBC that has been proposed is:

> . . .. . . hereditary lobular breast cancer is defined in this context by the presence of a pathogenic CDH1 variant in either an isolated individual with LBC, or a family with one or more LBC cases in first-degree or second-degree relatives, but no known DGC in either situation. By definition, families with HLBC are recategorized as Hereditary Diffuse Gastric Cancer if in the future, a case of diffuse gastric cancer is found.

Irrespective of whether HLBC will prove to be a truly 'independent' familial cancer syndrome—where for some, as yet unknown, reason members of these families never get diffuse gastric cancer—the management of HLBC families remains challenging. The finding of clinically occult microscopic foci of signet-ring cell gastric cancer in stomachs from women found to have a *CDH1* pathogenic variant (PV) after a diagnosis of LBC, who then underwent prophylactic gastroscopy, raises the possibility that it is all one syndrome 'HDGC' with variable penetrance for LBC and DGC [3].

3.2 History of HDGC Guidelines

For over 20 years, the International Gastric Cancer Linkage Consortium (IGCLC), a group of scientists, clinicians and family representatives working in HDGC, meet 3–5 yearly to review the literature and reach consensus on management of HDGC. Updated guidelines have been published after each meeting. Since early on, it was known there was an increased risk of LBC in HDGC [4]. As evidence accumulated with time, each successive IGCLC guideline contained more information on the management of LBC in HDGC patients.

Since its inception in 1999, the focus of the IGCLC has primarily been on gastric cancer risk management in HDGC, with less emphasis on LBC, in part because of the more rapid research developments in the former. Whilst the latest HDGC guideline covers risk management of LBC in HDGC and HLBC in more depth than previously, and there are other BC guidelines (e.g. NCCN, ESMO, EviQ) which cover management of patients with *CDH1* PV, there remains a real need for more research in HLBC, to facilitate LBC-specific individually tailored care in this unique group of patients.

3.3 Criteria for Screening the *CDH1* Gene in LBC

The finding of a germline *CDH1* pathogenic variation in an individual with a family history of LBC but *no* DGC was first described in 2008 [5]. Somewhat surprisingly, this was nearly 10 years after pathogenic variants in the *CDH1* gene (which codes for the cell-to-cell adhesion molecule E-cadherin) were first identified as the cause of HDGC in three large Maori families from Aotearoa-New Zealand [6].

The vast majority of HDGC families are identified due to an index case of DGC, not LBC. Some HDGC families are identified because the proband has LBC, and a DGC is subsequently identified in the family; however, it is curious how few patients with LBC and a family history of LBC have been found to have a *CDH1* pathogenic variant [7].

Clinical criteria for selection of LBC patients for *CDH1* pathogenic variant screening, in the absence of any personal or family of DGC, first appeared in the 2015 IGCLC guidelines [8]. The wording was 'testing could be considered' in bilateral LBC or a family history of $\geq$2 cases of LBCs $<$50 years. Recent years have seen these original criteria for testing *CDH1* in LBC evolve slightly. The current criteria are summarised in Fig. 3.1 and are simple to apply. The minor differences that have been made to the criteria for testing *CDH1* relate to the age when LBC is diagnosed in family members (see legend in Fig. 3.1).

3.4 Other Breast Cancer Predisposition Genes Associated with LBC

There are other breast cancer predisposition genes besides *CDH1*, that are associated with an increased risk of LBC. A large UK study in LBC patients showed PVs in *BRCA2* were more common than PVs in *CDH1* [9]. Depending on assessment by clinical genetics, a limited breast cancer panel test including *BRCA2, CHEK2, ATM,* and *PALB2* in addition to *CDH1* may be appropriate in a patient with LBC [10]. Globally, while there are some differences in acceptance of which genes should be on a breast cancer panel test—testing the above genes would be considered reasonable in a patient with LBC meeting criteria for *CDH1* testing. It is notable that PVs in the *BRCA1* gene do not appear to be associated with LBC [10].

3.5 LBC and Family History But No PV Detected

In most individuals who meet the criteria for *CDH1* testing, no PV will be found. This is referred to as an 'inconclusive' result because whilst no abnormality has been found in the *CDH1* gene (nor any other genes if a limited breast cancer panel test was done), it remains possible there is an unknown gene (or combination of genes) causing the increased breast cancer risk in the family. An 'inconclusive' result is the most likely outcome in genetic testing and is explained as part of the genetic counselling process fundamental to informed consent.

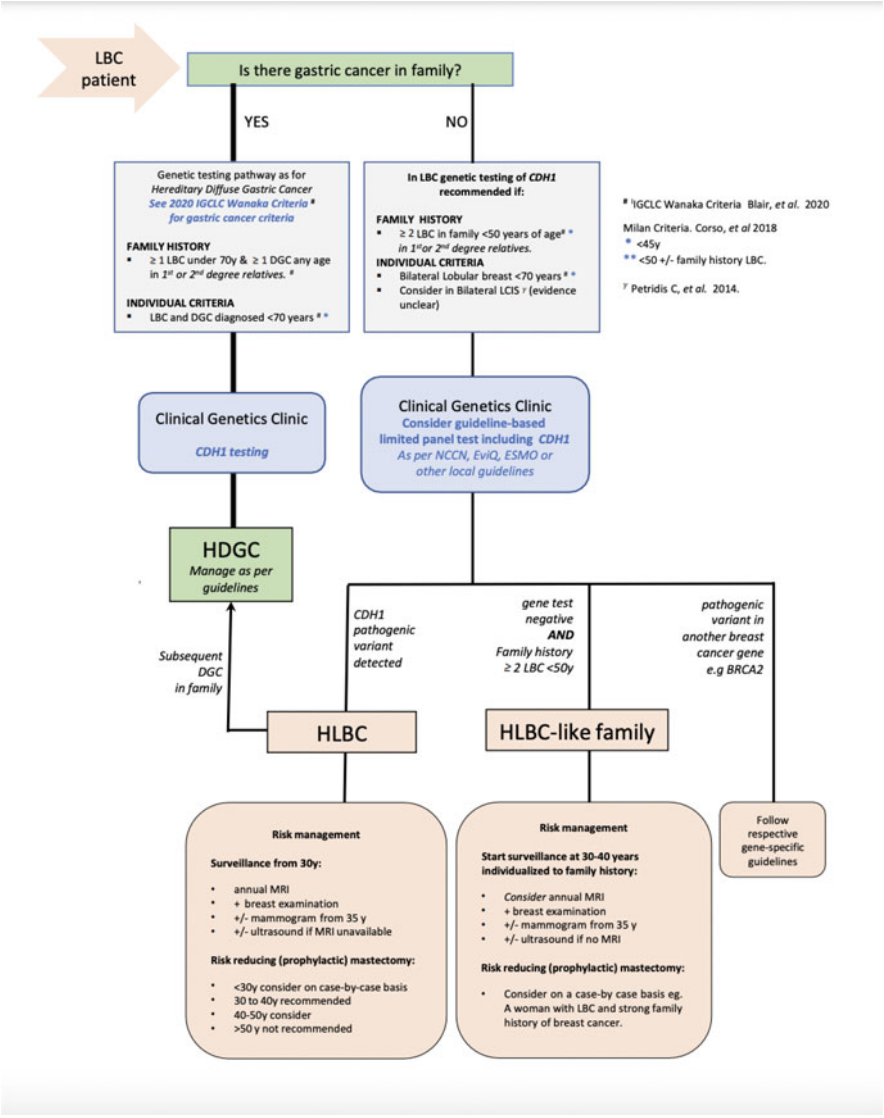


Fig. 3.1 Hereditary lobular breast cancer: diagnosis and management. Note: Some guidelines recommend all women with breast cancer be 'offered' the option of genetic testing and there has been a rise in panel testing in breast cancer. Other breast cancer predisposition genes associated with LBC are: *BRCA2* and *ATM* (associated with high risk of LBC, OR $>$ 4), *CHEK2* and *PALB2* (associated with moderate risk of LBC, OR $=$ 2–4). Pathogenic variants in *BRCA1* do not appear associated with LBC. The frequency of pathogenic variants in a population-based cohort of LBC was 5–6%, similar to IC-NST

Patients who meet the criteria for *CDH1* testing, in whom no PV is found (in either *CDH1* or another LBC-associated gene), need to have risk management recommendations made after a risk-estimate based on assessment of their personal and family history. Mirroring the term 'HDGC-like' coined by the IGCLC to identify patients with DGC who meet the criteria for *CDH1* testing, in whom no PV is found, the term 'HLBC-like' is suggested for this subset of women. Having clear classification of different sub-sets of patients with LBC will help optimise individualised care and facilitate research.

3.6 Classification and Pathogenesis

Greater than 70% of breast cancers are classified as invasive carcinoma-no special type (IC-NST), a term now used in place of the older 'ductal breast cancer', as per the WHO classification system. LBC is the most common of the 'special types' of breast cancer, making up approximately 10–15% of all breast cancers. High hormone receptor positivity and low proliferation index are typical of LBC [11].

Accurate pathological classification of breast cancer is an essential first step to facilitate identification of HLBC families. The era of relying solely on lobular-type morphology on H&E stained slides to do this has long passed. One of the characteristic features of invasive LBC is the loss of E-cadherin expression and function in about 90% of cases [12]. But E-cadherin immunohistochemistry (ICH) can show aberrant staining, leading to the misdiagnosis of a LBC as a IC-NST. The important role of p120 IHC in the classification of LBC in this situation is discussed in detail elsewhere.

A good illustration of the fact that phenotypic clues from histological classification can provide hints to potential genotype is the fact that *BRCA2* mutations are associated with both IC-NST and LBC, whereas *CDH1* pathogenic variants are uniquely associated with LBC and pathogenic variants in BRCA1 do not appear to be associated with LBC.

The presence of an inherited *CDH1* pathogenic variant and subsequent acquisition of a second-hit causes *CDH1* inactivation and loss of E-cadherin expression. This loss or deregulation of E-cadherin function results in decreased cell-cell adhesion and increased cell proliferation. E-cadherin-deficient cells accumulate in the lobules giving rise to the spectrum of changes referred to as 'lobular intraepithelial neoplasia' (atypical lobular hyperplasia, lobular carcinoma in situ). Over time, if in situ cells get further genetic damage, they can gain the potential to invade the basement membrane and surrounding breast tissue, and progress into an invasive lobular carcinoma [1].

Firstly, Benusiglio in 2013 and then Petridis in 2014, identified new *CDH1* germline pathogenic variants in women with LBC or LCIS but without a family history of gastric carcinoma [13, 14]. Subsequently, other *CDH1* mutations have been identified with a frequency of about 3% in the screened population [15]. The latest review of the literature reported a total of 34 *CDH1* pathogenic variants identified to date in LBC or BC (without definition for lobular subtype) in 28 families

identified by screening to have the mutated gene [16]. To date, the overall mutation frequency appears low, and HLBC is therefore a relatively rare breast cancer predisposition syndrome.

3.7 Conclusion

HLBC is a newly defined entity where there is a predisposition to lobular type breast cancer related to inheritance of a *CDH1* pathogenic variant, without any gastric cancer in the family. It remains unclear whether HLBC families will ultimately prove to be part of the HDGC syndrome and only with time will it become clear whether cases of gastric cancer will develop in HLBC families, or not. Likely, not all HLBC families will be at equal risk of developing DGC, due to both environmental factors and other inherited genetic influences. Given the small number of HLBC families identified to date, the lifetime risk of developing LBC in HLBC is as yet unknown, but potentially is similar to that seen in women from HDGC families.

It is hoped that by increasing awareness of HLBC as an entity, this will increase the diagnosis of this rare subgroup of breast cancer patients. Obviously, this starts with the appropriate selection of LBC patients for testing for PVs in *CDH1* and other LBC-associated breast cancer predisposition genes. The rarity of HLBC means collaboration is key to advancing research in LBC and HLBC. The emerging paradigm of HLBC provides a unique opportunity to carry out research to improve LBC-specific diagnosis, investigation and treatment.

References

1. Corso G, Figueiredo J, La Vecchia C et al (2018) Hereditary lobular breast cancer with an emphasis on E-cadherin genetic defect. J Med Genet 55(7):431–441. https://doi.org/10.1136/jmedgenet-2018-105337
2. Blair VR, McLeod M, Carneiro F et al (2020) Hereditary diffuse gastric cancer: updated clinical practice guidelines. Lancet Oncol 21(8):e386–e397. https://doi.org/10.1016/S1470-2045(20)30219-9
3. Jacobs MF, Dust H, Koeppe E et al (2019) Outcomes of endoscopic surveillance in individuals with genetic predisposition to hereditary diffuse gastric cancer. Gastroenterology 157(1):87–96. https://doi.org/10.1053/j.gastro.2019.03.047
4. Pharoah PD, Guilford P, Caldas C et al (2001) Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. Gastroenterology 121(6):1348–1353. https://doi.org/10.1053/gast.2001.29611
5. Masciari S, Larsson N, Senz J et al (2007) Germline E-cadherin mutations in familial lobular breast cancer. J Med Genet 44:726–731. https://doi.org/10.1136/jmg.2007.051268
6. Guilford P, Hopkins J, Harraway J et al (1998) E-cadherin germline mutations in familial gastric cancer. Nature 392(6674):402–405. https://doi.org/10.1038/32918
7. Schrader KA, Masciari S, Boyd N et al (2011) Germline mutations in CDH1 are infrequent in women with early-onset or familial lobular breast cancers. J Med Genet 48(1):64–68. https://doi.org/10.1136/jmg.2010.079814

8. van der Post RS, Vogelaar IP, Carneiro F et al (2015) Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline *CDH1* mutation carriers. J Med Genet 52(6): 361–374. https://doi.org/10.1136/jmedgenet-2015-103094
9. Petridis C, Arora I, Shah S et al (2019) Frequency of pathogenic germline variants in CDH1, BRCA2, CHEK2, PALB2, BRCA1, and TP53 in sporadic lobular breast cancer. Cancer Epidemiol Biomark Prev 28(7):1162–1168. https://doi.org/10.1158/1055-9965.EPI-18-1102
10. Yadav S, Hu C, Nathanson KL et al (2021) Germline pathogenic variants in cancer predisposition genes among women with invasive lobular carcinoma of the breast. J Clin Oncol 39(35): 3918–3926. https://doi.org/10.1200/JCO.21.00640
11. Iorfida M, Maiorano E, Orvieto E et al (2012) Invasive lobular breast cancer: subtypes and outcome. Breast Cancer Res Treat 133(2):713–723. https://doi.org/10.1007/s10549-012-2002-z
12. Corso G, Figueiredo J, De Angelis SP et al (2020) E-cadherin deregulation in breast cancer. J Cell Mol Med 24(11):5930–5936. https://doi.org/10.1111/jcmm.15140
13. Benusiglio PR, Malka D, Rouleau E et al (2013) CDH1 germline mutations and the hereditary diffuse gastric and lobular breast cancer syndrome: a multicentre study. J Med Genet 50(7): 486–489. https://doi.org/10.1136/jmedgenet-2012-101472
14. Petridis C, Shinomiya I, Kohut K et al (2014) Germline CDH1 mutations in bilateral lobular carcinoma in situ. Br J Cancer 110(4):1053–1057. https://doi.org/10.1038/bjc.2013.792
15. Corso G, Figueiredo J, Biffi R et al (2014) E-cadherin germline mutation carriers: clinical management and genetic implications. Cancer Metastasis Rev 33(4):1081–1094. https://doi.org/10.1007/s10555-014-9528-y
16. Girardi A, Magnoni F, Vicini E et al (2022) CDH1 germline mutations in families with hereditary lobular breast cancer. Eur J Cancer Prev 31(3):274–278. https://doi.org/10.1097/CEJ.0000000000000688

Part II

Genetics

4 Genetic Counselling and Prevention in Families at High Risk for HDGC and Other Hereditary Syndromes

Cristina Zanzottera and Bernardo Bonanni

Abstract

Genetic counselling is a structured process that involves several steps: identification of the individuals in which it can be indicated to perform genetic testing in relation to their personal and family history, explanation to candidates of the meaning of genetic testing for themselves and family members, and, if the test is performed, communication of the result and discussion about its implications.

The diagnosis of a hereditary cancer predisposition syndrome allows to define personalized preventive options for at-risk individuals, healthy or affected by cancer. The discussion of preventive options requires a multidisciplinary approach, which considers current clinical practice recommendations, the patient's clinical status and wishes, but also family history.

The identification of at-risk individuals with a little or no family history of cancer represents a challenge in terms of oncological risk definition and clinical management.

4.1 Introduction

Most cancers are sporadic and only a minority of them can be considered hereditary, i.e. linked to the presence of pathogenic or likely pathogenic germline variants (commonly referred to as mutations) in a single gene capable of conferring a significantly increased risk of developing the disease during life. It has been estimated that about 1–3% of gastric cancers and about 5–10% of breast cancers

C. Zanzottera · B. Bonanni (✉)
Division of Cancer Prevention and Genetics, European Institute of Oncology (IEO) IRCCS, Milan, Italy
e-mail: bernardo.bonanni@ieo.it

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_4

can be considered hereditary, although the genes currently known justify only a proportion of families with a history suggestive for high genetic risk [1–4].

To date, the diagnosis of a hereditary cancer predisposition syndrome is made on the basis of clinical criteria, that consider personal and/or family history, and molecular results, i.e. the finding of a germline mutation: hereditary breast and ovarian cancer (HBOC), caused by germline mutations in the *BRCA1* and *BRCA2* genes, is undoubtedly the most frequent and best-known cancer syndrome in the population [4], while rarer, but not less relevant, are conditions such as hereditary diffuse gastric cancer (HDGC) and hereditary lobular breast cancer (HLBC), related to germline mutations in the *CDH1* gene [5, 6].

As a rule, to select individuals who might benefit from a genetic evaluation, it is important to pay attention to situations such as:

- Early age at diagnosis.
- Family aggregation (i.e. more individuals in the same branch of the family affected by the same cancer).
- Presence, in the same individual or in more individuals of the same family branch, of specific malignant tumours or dysmorphisms, congenital malformations, skin changes and/or characteristic benign neoplasms.

The main hereditary cancer predisposition syndromes have specific clinical criteria for genetic testing, which can be easily retrieved in the literature and/or in the main guidelines (e.g. NCCN guidelines).

4.2 Hereditary Diffuse Gastric Cancer

Regarding HDGC, after the identification of the first *CDH1* mutation in Māori families characterized by aggregation for diffuse gastric cancer in 1998 [7], the International Gastric Cancer Linkage Consortium (IGCLC) defined selective clinical criteria for *CDH1* genetic testing: these criteria have been progressively revised in multidisciplinary expert meetings, lastly in 2019 [5]. Revisions became needed primarily due to the observation of the association between lobular breast cancer and germline *CDH1* mutations, even in the absence of a positive family history for gastric cancer. Furthermore, the increasing accessibility to genetic testing, particularly for breast cancer, has led to identification of *CDH1* mutations in individuals with less significant family history of cancer [5, 6, 8]. Currently, *CDH1* genetic testing is recommended when one of the following criteria has been met [5]:

Family criteria:

1. ≥2 cases of gastric cancer in the family regardless of age, with at least one diffuse gastric cancer (DGC).
2. ≥1 case of DGC at any age and ≥1 case of lobular breast cancer (LBC) <70 years, in different family members.
3. ≥2 cases of LBC in family members <50 years of age.

Individual criteria:

1. DGC at age < 50 years
2. DGC at any age in individuals of Māori ethnicity
3. DGC at any age in individuals with a personal or family history (first-degree relative) of cleft lip or cleft palate
4. History of DGC and LBC, both diagnosed at age <70 years
5. Bilateral LBC, diagnosed <70 years
6. Gastric in situ signet ring cells or pagetoid spread of signet ring cells in individuals <50 years of age

4.3 Other Hereditary Cancer Syndromes

Consequently, the collection of personal and family history, defined as the description of the genetic relationships and medical history of a family, is the first step in the genetic counselling process [5, 6]. Data collection can be performed before or during the consultation: the most common tool used is a family medical history questionnaire, administered prior to counselling in order to request the necessary information and documentation [6]. Several studies have demonstrated that self-reported family history is often inaccurate and, where possible, cancer cases should be confirmed by medical reports [9–11]. Not all cancers are syndromic and different histologies of a cancer can lead to suspicion of different hereditary cancer predisposition syndromes: for instance, non-lobular breast cancer and intestinal-type gastric cancer are not recognized as part of HDGC [5], while they might be consistent with a diagnosis of other conditions, such as HBOC and Lynch syndrome, respectively. Gastric cancer can be involved in other syndromes besides HDGC, such as Peutz-Jeghers syndrome (PJS), juvenile polyposis syndrome (JPS), Lynch syndrome (LS), Li-Fraumeni syndrome (LFS), classical familial adenomatous polyposis (FAP) and gastric adenocarcinoma and proximal polyposis syndrome (GAPPS), *MUTYH*-associated adenomatous polyposis (MAP) and also HBOC, even if the risk, when estimated, is generally low: we should also mention a syndromic condition whose genetic causes have not yet been identified, namely familial intestinal gastric cancer (FIGC) [1, 2].

Therefore, accurate family history data collection with histological confirmation is often crucial to evaluate the indication to perform genetic testing and to identify the most appropriate candidate [5, 6]. Genetic testing is generally performed initially in an individual with cancer, who in the majority of cases is the member of the family with the earliest age of onset of cancer. Predictive genetic testing should be offered to adults, able to formulate an informed consent; in the case of an affected minor patient, the consent of both parents must be discussed and obtained to proceed with genetic testing. When there are no affected individuals living and/or available to carry out genetic investigations, but the family meets the clinical criteria, genetic testing in a healthy individual can be considered.

An informed consent implies that the individual to whom the test is proposed is informed about the possible clinical manifestations of a genetic condition, any

treatments available and/or the strategies to prevent it and the risk of recurrence and transmission in family members. Therefore, genetic counselling is a complex medical process that involves biological, medical and psychological aspects, before and after genetic testing.

4.4 Genetic Testing Evaluation

The delivery and discussion of the result of genetic testing is another fundamental step of genetic counselling process because the different results allow to define the oncological genetic risk of the patient and, in some cases, also the subsequent therapeutic options. In particular, in individuals at high genetic risk, i.e. carriers of germline mutations, it is possible to offer customized preventive options, including clinical-instrumental surveillance and, where applicable, chemoprevention and risk reduction surgery.

Furthermore, the identification in an individual of a mutation allows to offer to interested relatives the possibility of a targeted test to confirm their carrier status ('cascade screening'), so as to identify other potentially at-risk family members to whom propose personalized prevention. Therefore, a predictive genetic testing involves not only the individual being tested but also his or her family and counselling should help patients to understand the importance of sharing their diagnosis to at-risk family members [5].

In healthy at-risk individuals, testing should be offered starting from the legal age of consent (generally 16–18 years). Testing of younger individual can be considered based on family history, i.e. in case of a documented family history of cancer at a young age, as can happen in conditions such as FAP, LFS and also HDGC [12–14].

Hereditary cancer predisposition syndromes are characterized by an increased lifetime risk of developing cancer, although an increased risk does not mean certainty of disease. The dimension of the risk is influenced by genetic background, lifestyle factors [2, 4] and, in some cases, the type of mutation [12, 13, 15, 16]. Consequently, penetrance of a mutation can be very variable between families and family history should be considered to estimate the risk of a single individual. For example, considering families meeting all the more stringent 2010 HDGC clinical criteria, the cumulative risk of gastric cancer in association with a *CDH1* mutation has been estimated to be up to 70% in men and 56% in women [8]. However, a report including families not fulfilling all the 2015 HDGC clinical criteria estimated a lower risk, 42% for male and 33% for women: the estimates were even lower considering only families with a little or no history of gastric cancer, 27% for male and 24% for women [17].

4.5 Surveillance

Breast is the organ most frequently involved in hereditary cancer predisposition syndromes, followed by ovaries, colon and endometrium; less frequently skin, stomach, pancreas, urinary tract, thyroid or others can be involved. Targeted surveillance is generally the primary strategy for prevention, while risk reduction surgery is considered only for some organs (e.g. breast, ovaries, uterus, colon, stomach), when the increased genetic risk and/or the scarce diagnostic-therapeutic possibilities justify the procedure. Prognosis and clinical condition of the patient (e.g. age, associated diseases, life expectancy) should also be considered.

Endoscopy, even when performed following Cambridge protocol by experienced endoscopists, has not yet reached full effectiveness in early diagnosis of DGC; therefore, *CDH1* mutation carriers should be advised to consider prophylactic total gastrectomy, possibly in early adulthood, especially in the presence of positive family history for gastric cancer. However, in families with no family history of DGC, e.g. HLBC families, the management of potential gastric risk is not straightforward and the indication to consider prophylactic total gastrectomy is debated, because of increased perioperative risks and prolonged recovery due to long-term sequelae. Consequently, multidisciplinary counselling is always required to comprehensively discuss benefits and risks of surveillance and risk-reducing surgery: the patient's choice to undergo prophylactic surgery must be aware and motivated so that he/she does not express regrets afterwards [2, 5].

High risk for breast cancer, i.e. greater than 40% in lifetime, is a common element in several hereditary cancer predisposition syndromes [2] and generally intensive surveillance through MRI is recommended. This risk could also be managed with risk-reducing mastectomy, in order to reduce the risk of developing cancer and cancer-related mortality. While risk-reducing mastectomy is now a widespread practice in women with HBOC, supported by literature data [18], there is not yet sufficient scientific evidence to systematically recommend risk-reducing mastectomy in *CDH1* female carriers. However, in selected cases, the surgical strategy could be discussed on the basis mainly of family history and in accordance with patients' wishes [5, 6].

4.6 Conclusion

Genetic counselling is a complex process and, in recent years, it has become even more so due to wider access of the population to genetic testing. More public awareness, suitability and cheapness of new technologies and consequent greater adoption of cancer gene panels allowed to identify a growing number of mutation carriers belonging to families with a little or no family history of cancer [19]. The management of prevention in these individuals represents a challenge and a territory worth further investigation.

References

1. Oliveira C, Pinheiro H, Figueiredo J et al (2015) Familial gastric cancer: genetic susceptibility, pathology, and implications for management. Lancet Oncol 16(2):e60–e70. https://doi.org/10.1016/S1470-2045(14)71016-2
2. Petrovchich I, Ford JM (2016) Genetic predisposition to gastric cancer. Semin Oncol 43(5):554–559. https://doi.org/10.1053/j.seminoncol.2016.08.006
3. Margolin S, Johansson H, Rutqvist LE et al (2006) Family history, and impact on clinical presentation and prognosis, in a population-based breast cancer cohort from the Stockholm County. Familial Cancer 5:309–321. https://doi.org/10.1007/s10689-006-7851-3
4. Kleibl Z, Kristensen VN (2016) Women at high risk of breast cancer: molecular characteristics, clinical presentation and management. Breast 28:136–144. https://doi.org/10.1016/j.breast.2016.05.006
5. Blair VR, McLeod M, Carneiro F et al (2020) Hereditary diffuse gastric cancer: updated clinical practice guidelines. Lancet Oncol 21(8):e386–e397. https://doi.org/10.1016/S1470-2045(20)30219-9
6. Corso G, Figueiredo J, La Vecchia C et al (2018) Hereditary lobular breast cancer with an emphasis on E-cadherin genetic defect. J Med Genet 55(7):431–441. https://doi.org/10.1136/jmedgenet-2018-105337
7. Guilford P, Hopkins J, Harraway J et al (1998) E-cadherin germline mutations in familial gastric cancer. Nature 392:402–405. https://doi.org/10.1038/32918
8. van der Post RS, Vogelaar IP, Carneiro F et al (2015) Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. J Med Genet 52(6):361–374. https://doi.org/10.1136/jmedgenet-2015-103094
9. Kerber RA, Slattery ML (1997) Comparison of self-reported and database-linked family history of cancer data in a case-control study. Am J Epidemiol 146:244–248. https://doi.org/10.1093/oxfordjournals.aje.a009259
10. Murff HJ, Spigel DR, Syngal S (2004) Does this patient have a family history of cancer? An evidence-based analysis of the accuracy of family cancer history. JAMA 292:1480–1489. https://doi.org/10.1001/jama.292.12.1480
11. Flint ND, Bishop MD, Smart TC et al (2021) Low accuracy of self-reported family history of melanoma in high-risk patients. Fam Cancer 20(1):41–48. https://doi.org/10.1007/s10689-020-00187-0
12. Yen T, Stanich PP, Axell L, et al (1998) APC-associated polyposis conditions. In: GeneReviews® [Internet]. University of Washington, Seattle; 1993–2022. 1998 Dec 18 [updated 2022 May 12]. Bookshelf ID: NBK1345
13. Frebourg T, Lagercrantz SB, Oliveira C et al (2020) Guidelines for the li-Fraumeni and heritable TP53-related cancer syndromes. Eur J Hum Genet 28(10):1379–1386. https://doi.org/10.1038/s41431-020-0638-4
14. Gullo I, Devezas V, Baptista M et al (2018) Phenotypic heterogeneity of hereditary diffuse gastric cancer: report of a family with early-onset disease. Gastrointest Endosc 87:1566–1575. https://doi.org/10.1016/j.gie.2018.02.008
15. Shimelis H, Mesman RLS, Von Nicolai C et al (2017) BRCA2 hypomorphic missense variants confer moderate risks of breast cancer. Cancer Res 77(11):2789–2799. https://doi.org/10.1158/0008-5472.CAN-16-2568

16. Moghadasi S, Meeks HD, Vreeswijk MP et al (2018) The BRCA1 c.5096G>A p.Arg1699Gln (R1699Q) intermediate risk variant: breast and ovarian cancer risk estimation and recommendations for clinical management from the ENIGMA consortium. J Med Genet 55(1):15–20. https://doi.org/10.1136/jmedgenet-2017-104560
17. Roberts ME, Ranola JMO, Marshall ML et al (2019) Comparison of CDH1 penetrance estimates in clinically ascertained families vs families ascertained for multiple gastric cancers. JAMA Oncol 5(9):1325–1331. https://doi.org/10.1001/jamaoncol.2019.1208
18. Jakub JW, Peled AW, Gray RJ et al (2018) Oncologic safety of prophylactic nipple-sparing mastectomy in a population with BRCA mutations. JAMA Surg 153:123. https://doi.org/10.1001/jamasurg.2017.3422
19. Lowstuter K, Espenschied CR, Sturgeon D et al (2017) Unexpected CDH1 mutations identified on multigene panels pose clinical management challenges. JCO Precis Oncol 1:1–12. https://doi.org/10.1200/PO.16.00021

CTNNA1, a New HDGC Gene: Inactivating Mechanisms and Driven Phenotypes

5

Silvana Lobo, Paulo S. Pereira, Patrick R. Benusiglio, and Carla Oliveira

Abstract

This chapter focuses on *CTNNA1*, the second gene to be acknowledged as a hereditary diffuse gastric cancer (HDGC) predisposing gene. *CTNNA1* loss of function was first found in a family meeting HDGC criteria in 2013. *CTNNA1*

S. Lobo
i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal

International Doctoral Programme in Molecular and Cellular Biotechnology Applied to Health Sciences (BiotechHealth) from Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal

P. S. Pereira
i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

IBMC – Instituto de Biologia Molecular e Celular, Porto, Portugal

P. R. Benusiglio
Unité fonctionnelle d'Oncogénétique clinique, Département de Génétique, Groupe Hospitalier Pitié-Salpêtrière, AP-HP, Sorbonne Université, Paris, France

Chirurgie générale et digestive, Hôpital Saint-Antoine, AP-HP, Sorbonne Université, Paris, France

C. Oliveira (✉)
i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal

FMUP – Faculty of Medicine of the University of Porto, Porto, Portugal
e-mail: carlaol@i3s.up.pt

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_5

loss of function germline variants affect a smaller fraction of HDGC families, as compared to *CDH1* variants. *CTNNA1* missense germline variants predispose specifically to macular dystrophy patterned 2, an autosomal dominant eye disorder.

Throughout this book chapter, we will deepen the knowledge on *CTNNA1* and αE-catenin protein and their involvement in HDGC, we will review *CTNNA1* germline variants distribution and association with disease phenotypes, and describe *CTNNA1*-related mechanisms underlying tumor formation and development in sporadic cancer. In particular, we will address predisposition related to *CTNNA1* germline pathogenic variants and development of diffuse gastric cancer, *CTNNA1* germline likely pathogenic variants and development of breast cancer of unknown histotype; data available on *CTNNA1* germline variants and lobular breast cancer; predisposition related to *CTNNA1* germline missense variants, classified as variants of unknown significance for HDGC, and development of macular dystrophy patterned 2; the importance of αE-catenin to connect and stabilize the adherens junction complex and the actin cytoskeleton; the multiple interactions of αE-catenin with different proteins and regulation of several signaling pathways; and the role of αE-catenin dysregulation in different types of sporadic cancer.

5.1 Introduction

Currently, there are two genes, for which inactivating germline variants predispose for HDGC, *CDH1* and *CTNNA1*, which encode for E-cadherin and αE-catenin, respectively [1]. Germline *CDH1* inactivation is the main cause of HDGC and has been known for more than 20 years [2, 3], while *CTNNA1* loss of function is a more recent discovery and affects a smaller fraction of HDGC families [1, 4, 5].

Catenins are a group of proteins that associate with the classic cadherins, which are Ca^{2+}-dependent proteins involved in cell-cell adhesion and are found in adherens junctions from well-polarized cells, such as epithelial cells [6–10].

The catenin family of proteins includes β-catenin, α-catenin, plakoglobin, and p120-catenin. From these, α-catenin differentiates itself by its lack of an Armadillo group in its composition and by its binding to both the adherens junctions and the cytoskeleton. α-catenins comprise αE-catenin, αN-catenin, and αT-catenin, which are encoded by *CTNNA1*, *CTNNA2*, and *CTNNA3* genes, respectively [11].

In this book chapter, we will deepen the knowledge regarding *CTNNA1*/αE-catenin and its role in HDGC, by assembling state-of-the-art information regarding gene and protein functions. We will also review the distribution of *CTNNA1* germline variants and their associated phenotypes and describe currently known mechanisms underlying tumor formation and development.

5.2 Brief Overview on *CTNNA1*/αE-catenin

The human *CTNNA1* gene is located on chromosome 5 (5q31.2) and encodes the epithelial α-catenin (αE-catenin). Its expression was originally associated with epithelial cells only, however, it is currently known that αE-catenin is expressed in most cell types [12]. The human canonical *CTNNA1* transcript (ENST00000302763.12) comprises 18 exons, 17 of which are coding and one 5′ non-coding exon. Furthermore, αE-catenin (NM_001903) protein is composed of 906 amino acids, with a molecular weight of 102 kDa [13–17]. αE-catenin is entirely cytoplasmic and holds four main domains on its architectural structure: a N-terminal β-catenin binding domain that also functions as the β-catenin/αE-catenin dimerization domain; a Vinculin binding domain; a regulatory M fragment with which several proteins interact; and a C-terminal domain that binds F-actin to regulate the actin cytoskeleton (AC) (Fig. 5.1). Besides being binding partners, Vinculin and αE-catenin present high homology at the amino acid sequence level [8, 10, 13]. Vinculin is important for cell-cell and cell-matrix adhesion and regulates F-actin by directly binding it from the cytoskeleton to the cell membrane, stimulating its polymerization and recruiting actin remodeling proteins [18–20]. Furthermore, αE-catenin is present in cells in two different conformations: monomers and homodimers. The different conformations present in cells bind to different proteins and, consequently, have different functions [21].

5.3 αE-catenin Connects the Adherens Junction Complex with the Actin Cytoskeleton

αE-catenin is the protein responsible for connecting the adherens junction complex (AJC) to the AC in human epithelial cells (Fig. 5.1) and, therefore, αE-catenin normal expression is extremely important for proper maintenance of both AJC and AC [7, 9, 14, 15, 22, 23].

5.3.1 αE-catenin and the Adherens Junction Complex

Cell-cell adhesion is the key feature for tissue development in vertebrates and invertebrates and involves specialized intercellular junctions, namely adherens junctions and their associated molecules from the cadherin and catenin families [14, 22, 24, 25]. The AJC is typically found in epithelial tissues, however, it is also present in fibroblasts, cardiac muscle, and neurons [26]. It is the AJC that enables epithelial cells to organize into monolayers, triggering several important features of epithelial tissues, such as tissue integrity and polarity, barrier function, cell shape and movement [22, 24, 27–29]. Furthermore, formation of the AJC is mandatory for proper epithelial organization in early stages of embryonic development and its regulation is extremely important to reorganize cells during embryogenesis, tissue

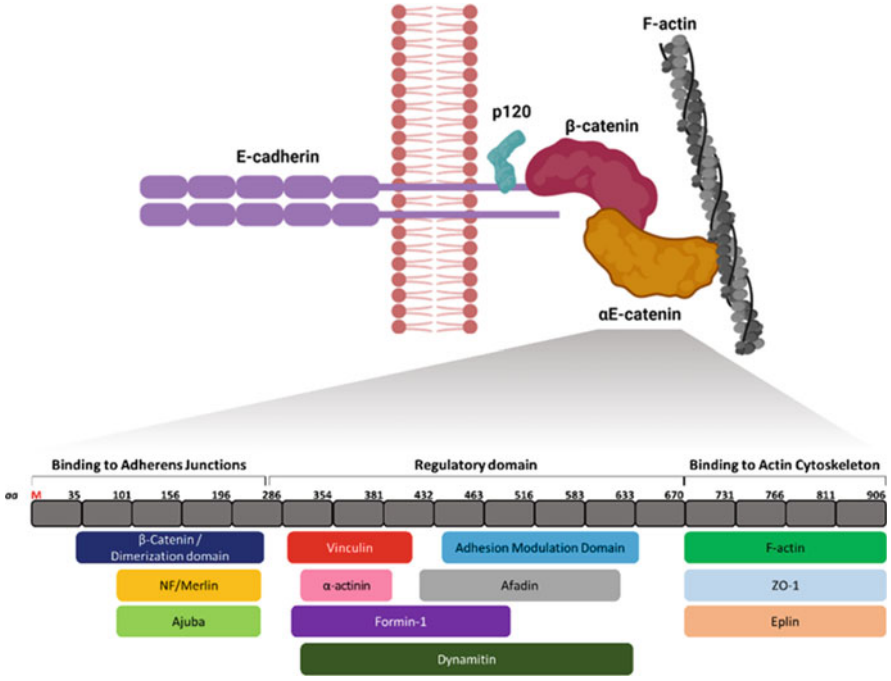


Fig. 5.1 αE-catenin interactions in epithelial cells. *Upper panel*, schematic representation of the connection of the adherens junction complex and the actin cytoskeleton through αE-catenin. Briefly, E-cadherin molecules bind to each other through their extracellular domain, while the cytoplasmic domain connects to β-catenin and p120-catenin. β-catenin, in turn, binds to the N-terminal domain of αE-catenin. The C-terminal domain of αE-catenin binds to the actin filaments of the actin cytoskeleton. This connection is not proven to be direct, hence, the most accepted model being the indirect/allosteric binding of αE-catenin to both complexes. In this model, αE-catenin monomers bind to β-catenin, creating αE-catenin/β-catenin heterodimers, while αE-catenin homodimers bind the actin filaments. *Lower panel,* schematic representation of proteins interacting with αE-catenin, and respective αE-catenin binding domains. These interactions, which are located in specific protein domains, enable stabilization of the adherens junction complex and the actin cytoskeleton

homeostasis, and wound healing, through the epithelial to mesenchymal transition [15, 22, 28, 29].

The principal component proteins of the epithelial AJC are E-cadherin, β-catenin, αE-catenin, and p120-catenin. E-cadherin forms homophilic contacts with other E-cadherin molecules on adjacent cells through its extracellular membrane. Then, the C-terminal region of E-cadherin binds to the 12 Armadillo repeats of β-catenin in the cytoplasm. This is important for stabilizing the unstructured cytoplasmic E-cadherin domain, preventing its degradation [15, 30, 31]. A 1:1:1 stoichiometric complex is formed with the binding of the N-terminal domain of αE-catenin to β-catenin [15, 32]. It has been proposed that αE-catenin builds the bridge to the AC. While the three abovementioned proteins are key players to regulate cell-cell

adhesion, p120-catenin regulates the AJC itself. p120-catenin binds to E-cadherin juxta-membrane domain which, in turn, stabilizes E-cadherin, preventing its internalization [15, 22, 28, 29, 33] (Fig. 5.1).

5.3.2 αE-catenin and the Actin Cytoskeleton

The F-actin cytoskeleton is composed of actin filaments that assemble into complex structures, such as bundles and networks. The actin filaments are composed of monomeric, globular actin molecules that can polymerize into filaments [34–37]. The rapid building, dismantling, and reorganization of the actin filaments is imperative for several cell aspects, such as shaping, motility, and force generation [37, 38]. There are proteins, named actin-binding proteins, that facilitate the actin polymerization to form the filaments. Indeed, several of these proteins interact with αE-catenin. Formin, an αE-catenin interactor, accelerates the development of linear actin filaments [8, 12, 14, 37]. On the other hand, αE-catenin can inhibit the Arp2/3-dependent actin polymerization of branched actin filaments [8, 37].

After F-actin polymerization, the filaments assemble into bundles and branched actin networks, higher order structures that provide a stable cell shape and regulate biological function. Several proteins that interact or bind to αE-catenin facilitate F-actin bundlings, such as vinculin, α-actinin, and formin [8, 12, 14, 37]. Indeed, αE-catenin undergoes conformational changes that expose vinculin binding sites, which in turn, stabilizes the interaction with E-cadherin and the AC. This supports the growth of the AJC and is followed by increased F-actin density, which expands the ability of cells to withstand externally applied forces [18, 39–41] (Fig. 5.1).

5.3.3 Direct *vs* Indirect Binding of the Adherens Junction Complex to the Actin Cytoskeleton

Throughout the past decades, scientists have been trying to understand how does αE-catenin bind the AJC to the AC. Two main theories have been put forward. The first relies on αE-catenin as a physical linkage between cadherins and the AC, while the second theory is based on αE-catenin as an allosteric regulator of these two molecules [21, 42, 43]. The first theory was the most widely accepted, although this model lacks experimental support to prove the physical linkage. The simplest model is based on the fact that αE-catenin can bind to E-cadherin/β-catenin through its N-terminal domain and can also bind to the AC, through its C-terminal domain, implying a physical linkage between the AJC and the AC through the direct binding of both molecules to αE-catenin. However, this model is hard to reconcile with the characterization of the allosteric regulation of αE-catenin. In the N-terminal domain of αE-catenin there is a binding site to β-catenin, which is common to the αE-catenin homodimerization site. This implies that the binding of αE-catenin to β-catenin or αE-catenin homodimerization are mutually exclusive events [21, 44]. αE-catenin exists in cells as both a monomer and a homodimer and these different

conformations are associated with distinct functions [21]. The allosteric regulation theory relies on these different αE-catenin conformations in cells. The monomeric αE-catenin binds to β-catenin, which in turn binds to E-cadherin with an increased affinity. Nevertheless, this monomeric αE-catenin from the cadherin-catenin complex has very low affinity to bind to F-actin [13]. In fact, work conducted by Drees and Yamada [23, 42] successfully demonstrated that αE-catenin cannot bind actin in the presence of β-catenin, which may be due to conformational changes occurring in the monomeric αE-catenin when it binds to the cadherin-catenin complex. Therefore, a more complex model was developed, where the physical linkage is indirect, occurring through the binding of E-cadherin/β-catenin to αE-catenin and the interaction of αE-catenin to actin-binding proteins vinculin, α-actinin, afadin, ZO-1, and formin that subsequently bind to F-actin [21]. A study conducted by Abe and colleagues [45] successfully demonstrated that an actin-binding protein, EPLIN, was responsible for the indirect physical linkage of E-cadherin/β-catenin to the AC, through the ligation of αE-catenin to this molecule. The authors state that in the absence of EPLIN, the linkage is not formed causing changes in adherens junctions conformations.

5.4 *CTNNA1* Germline Variants Predispose to HDGC

5.4.1 Brief Overview of HDGC

Hereditary diffuse gastric cancer (HDGC) is an autosomal dominant cancer syndrome that predisposes to diffuse gastric cancer (DGC) and invasive lobular breast cancer (LBC) development [1, 46]. *CDH1* germline truncating variants are found in 10%–40% of cases suggestive of HDGC [3, 46]. Several studies have determined that the cumulative risk of developing gastric cancer (GC) in *CDH1* variant carriers varies from 37% to 64% in men and 24% to 47% in women. Moreover, women have an additional cumulative risk of developing breast cancer (BC) from 39% to 55% [1, 46–49] and a combined risk of developing BC and GC of 78% by age 80 [46].

Knowledge acquired on the last two decades on *CDH1* germline variant carriers has been useful to update clinical criteria guiding *CDH1* genetic testing and respective guidelines on how to manage *CDH1*-positive HDGC families [1, 50–52].

Through the last years, several candidate genes, like *CTNNA1* [5, 46, 53], *PALB2* [46, 54], or *MAP3K6* [55], have been reported in early-onset and familial DGC patients. Nevertheless, *CTNNA1* is the only gene to be widely accepted as a true HDGC predisposing gene. The first family carrying a *CTNNA1* germline variant was identified in 2013, where several family members presented early-onset DGC and clearly fulfilled HDGC criteria [5]. Since then, 41 families bearing *CTNNA1* germline variants and presenting cancer phenotypes, either the classical HDGC-associated phenotypes or others, were identified [4].

5.4.2 HDGC Guidelines for *CTNNA1* Germline Variant Carriers

CTNNA1 was officially considered an HDGC predisposing gene, alongside *CDH1*, in the HDGC guidelines published in 2020 [1]. According to the guidelines (Table 5.1), carriers of germline *CTNNA1* pathogenic variants (PV) are recommended to undergo annual endoscopy screening with multiple random biopsies, in accordance with the Cambridge protocol. Prophylactic total gastrectomy (PTG) should also be discussed, as illustrated by the observation of pT1a foci in asymptomatic carriers [56]. However, less emphasis should be put on PTG, as compared to carriers of *CDH1* PV, since data currently available on *CTNNA1* families are, at this stage, limited.

5.4.3 Clinical Classification of *CTNNA1* Germline Variants and Distribution in HDGC Ascertained and Non-ascertained Cohorts

The proper clinical classification of *CDH1* germline variants has been pivotal to identify actionable *CDH1* variants (pathogenic—PV and likely pathogenic—LPV) and, consequently, improve management of patients with HDGC. *CDH1* germline

Table 5.1 HDGC updated clinical practice guidelines [1]

HDGC updated clinical practice guidelines (Blair et al., 2020)	
Family criteria	
1	≥2 cases of gastric cancer in family regardless of age, with at least one diffuse gastric cancer (DGC)
2	≥1 case of DGC at any age, and ≥ 1 case of lobular breast cancer (LBC) at age < 70 years, in different family members
3	≥2 cases of LBC in family members <50 years of age
Individual criteria	
4	Isolated case of DGC at age < 50 years
5	DGC at any age in individuals of Māori ethnicity
6	DGC at any age in individuals with a personal or family history (first-degree relative) of cleft lip or cleft palate
7	History of DGC and LBC, both diagnosed at age < 70 years
8	Bilateral LBC, diagnosed at age < 70 years
9	Gastric in situ signet ring cells or pagetoid spread of signet ring cells in individuals <50 years of age

Clinical criteria were updated from the HDGC clinical guidelines from 2015 [52] to include more families with HDGC-related phenotypes. It is recommended to perform genetic testing for *CDH1* mutation if one of the following criteria is met and cancer diagnosis is confirmed. If more than one cancer is confirmed, at least one should have histology confirmed. If possible, other relevant cancer phenotypes should be confirmed. Individuals fulfilling criteria that are negative for a *CDH1* mutation should be subsequently considered for *CTNNA1* analysis

Individuals fulfilling these criteria proving negative for *CDH1* actionable variants should be subsequently considered for *CTNNA1* sequencing

variants clinical classification has extensively improved upon development of specific variant curation guidelines by the American College of Medical Genetics and Genomics (ACMG), the Association for Molecular Pathology (AMP), the National Institute of Health NIH funded Clinical Genome Resource (ClinGen), and the *CDH1* variant curation expert panel (*CDH1* VCEP) [57, 58]. In contrast, no specific variant curation guidelines exist so far to classify *CTNNA1* variants. Consequently, it is difficult to properly address *CTNNA1* variants in the clinical setting and disclose their actionability in HDGC. Recently, we used the *CDH1*-specific guidelines to clinically classify published *CTNNA1* germline variants [4]. Even though both genes are tumor suppressor genes, partners in the AJC, and both present autosomal dominant patterns of inheritance in HDGC, the insufficient clinical data on *CTNNA1* variant carriers may have slightly biased the variant classification of *CTNNA1* variants [4]. Our systematic review [4] gathered 41 families from six original articles bearing *CTNNA1* germline variants [5, 46, 53, 56, 59, 60]. From these, the cohorts by Clark et al. and Shirts et al. were large cohorts, where the main goal was to genetically screen individuals with personal and/or family cancer history, regardless of the type of cancer. Therefore, in these two non-ascertained cohorts the finding of *CTNNA1* germline variants was incidental [59, 60]. On the other hand, the remaining four cohorts [5, 46, 53, 56] were smaller, included families and individuals fulfilling HDGC clinical criteria, and were ascertained for testing of *CTNNA1* germline variants, by fulfillment of clinical criteria for HDGC [1]. The frequency of *CTNNA1* germline variants in ascertained cohorts is significantly higher than in non-ascertained cohorts (2.1% vs. 0.036%), supporting *CTNNA1* as a HDGC predisposing gene.

5.4.4 Distribution of *CTNNA1* Germline Variants in HDGC and Non-HDGC Families

The 41 families bearing *CTNNA1* germline variants described in the literature [5, 46, 53, 56, 59, 60] presented 31 different variants [4]. The majority of these were considered truncating and predicted to have a high impact on protein structure and function (Fig. 5.2). Thirty-two percent of families carrying *CTNNA1* variants fulfilled HDGC clinical criteria, i.e., criteria warranting germline genetic testing; however, a large fraction of families did not [1]. The observation of cases carrying truncating variants, while not suggestive of HDGC, may be explained by missing clinical data regarding relatives, and incomplete DGC penetrance [46].

From the families meeting HDGC clinical criteria, almost all carried a pathogenic variant, specifically frameshift or nonsense variants. These variants were scattered across the gene and, in these families, a strong association with early-onset DGC was observed.

One cluster of variants (all truncating) was found to be located in the last exon of *CTNNA1*. Interestingly, these were found only in families lacking HDGC clinical criteria (Fig. 5.3). As in most genes, *CTNNA1* last exon and the 55 nucleotides upstream the last exon-exon junction is predicted to be a nonsense mediated mRNA

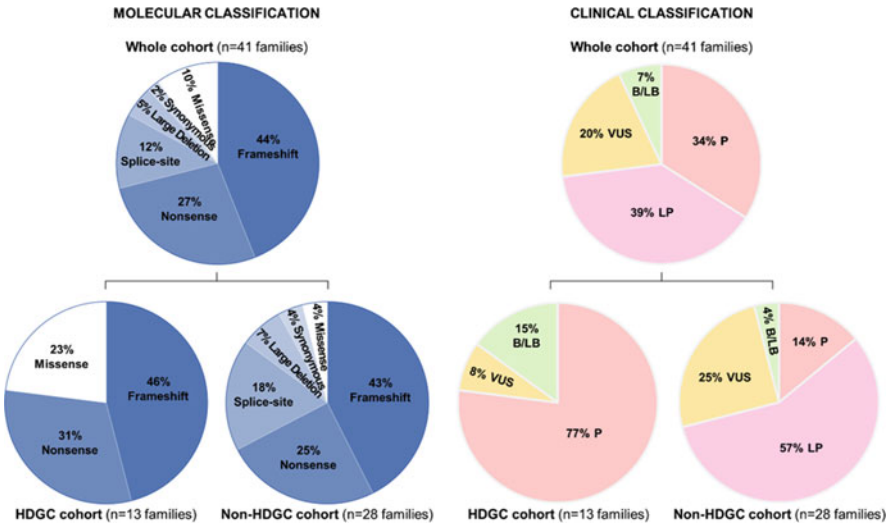


Fig. 5.2 Molecular and clinical classification of *CTNNA1* variants in families with and without HDGC. *Left panel*, Frequency analysis regarding the molecular classification of variants: total cohort (upper graph); HDGC and non-HDGC cohorts (lower graphs). *Right panel*, Frequency analysis regarding the clinical classification of *CTNNA1* variants: total cohort (upper graph); HDGC and non-HDGC cohorts (lower graphs). Clinical classification was performed using the ACMG/AMP *CDH1*-specific curation guidelines [57]. P—pathogenic; LP—likely pathogenic; VUS—variant of uncertain significance; LB—likely benign; B—benign

decay (NMD) incompetent region. As so, it is expected that a transcript with a truncating variant that produces a premature stop codon in this region most likely evades NMD, producing an abnormal αE-catenin protein still retaining residual function [61]. This was previously described for *CDH1* germline variant carriers [62, 63] and, if true for *CTNNA1*, it would support the establishment of a transversal NMD incompetent region for both HDGC predisposing genes, which would ultimately improve the clinical and molecular classification of *CTNNA1* germline variants.

5.4.5 Genotype-Phenotype Correlations in *CTNNA1* Germline Variant Carriers

In 2021, we published a genotype-phenotype correlation study using data from 105 individuals from 41 families carrying *CTNNA1* germline variants [5, 46, 53, 56, 59, 60], where 41 were probands and 64 were direct blood relatives [4]. Two major phenotypes stood up among all: DGC and BC. Almost all DGC cases were observed in families carrying PV and an early age of onset is observed in these cases, with a mean age of onset of ~40 years old and a standard deviation of 17 years old (~40 ± 17 years old) in probands. As for BC cases, most were present in families bearing *CTNNA1* LPV. In these families, the age of onset for BC cases was also

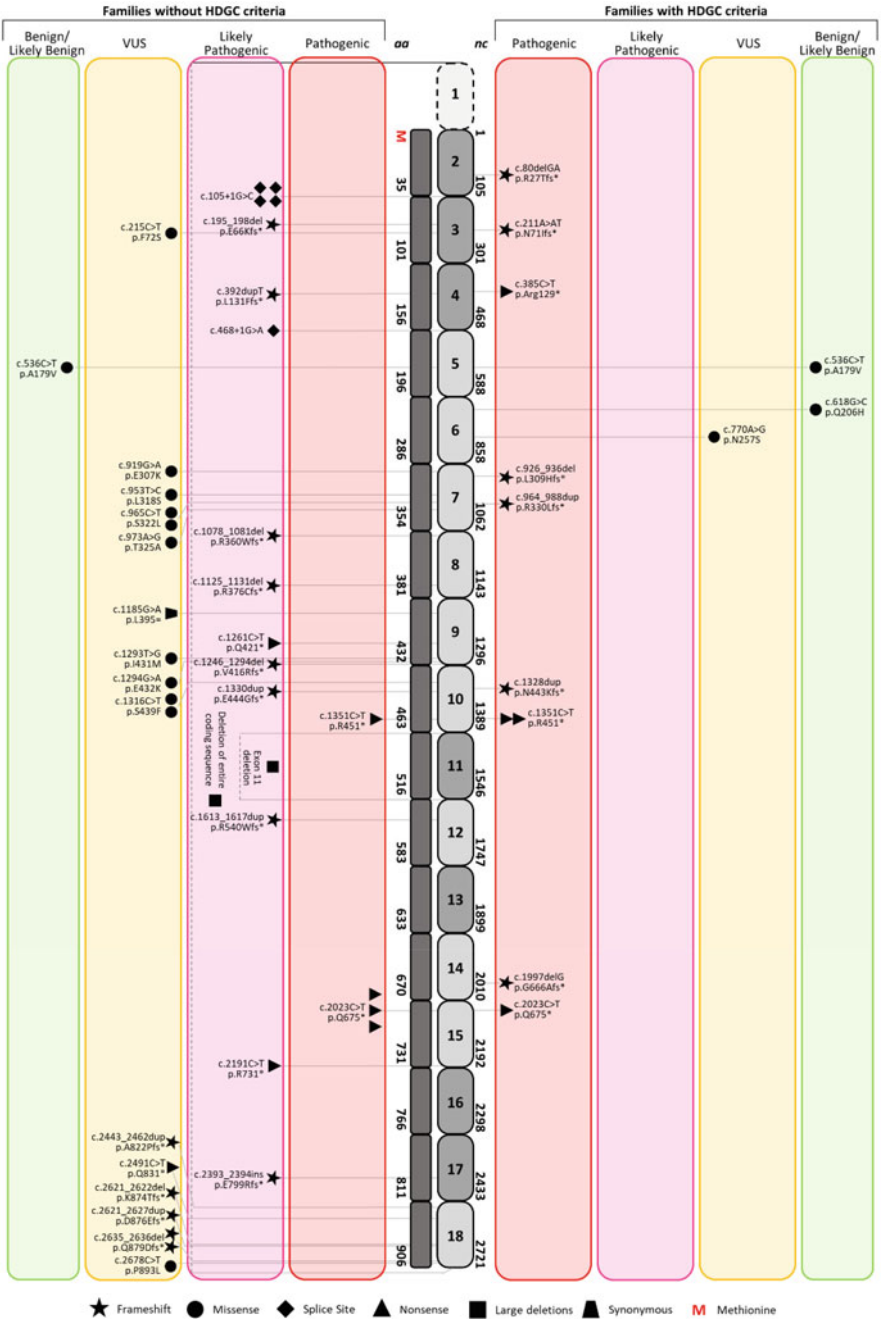


Fig. 5.3 Gene location of *CTNNA1* germline variants found in the literature. HDGC clinical criteria separate the two cohorts: families with HDGC criteria on the right and families without HDGC criteria on the left. The colored groups represent the clinical classification of *CTNNA1* variants according to the ACMG/AMP *CDH1* specific curation guidelines [57]: Red—pathogenic variants; Pink—likely pathogenic variants; Yellow—variants of uncertain significance; Green—

earlier than expected for the normal population, with a mean age of onset of ~57 ± 15 years old for probands, and ~51 ± 15 years old for relatives (Fig. 5.3).

While *CDH1* families bearing LPV and PV present a very similar disease spectrum, *CTNNA1* families carrying PV significantly differ from those carrying LPV. Most *CTNNA1* PV-carrier families fulfill HDGC clinical criteria, while no LPV-carrier families did (Fig. 5.4). Indeed, BC and DGC cases in *CTNNA1* germline variant carrier families are hardly observed in concomitance within the same family [4]. Contrarily to what is observed in *CDH1* variant carriers, *CTNNA1* LPV, classified according to the *CDH1*-specific ACMG/AMP guidelines for variant classification [57], may not reach 90% likelihood of pathogenicity, hence, only *CTNNA1* PV should be considered actionable in HDGC.

Furthermore, LBC cases are rare in *CTNNA1* germline variant carrier families, which may indicate a weaker association between *CTNNA1* and LBC, compared to *CDH1* [1]. Another possibility is that BC in *CTNNA1* PV-carriers is actually of the lobular type, but that specific information was not provided in previous publications. There is no evidence at this stage for an association of *CTNNA1* PV or LPV with ductal (no special type) breast cancer (DBC). Given the scarce information of the relationship between BC predisposition and *CTNNA1* germline variants, clinical management of BC in families carrying *CTNNA1* germline variants is currently evaluated on a case-by-case basis, according to the International Gastric Linkage Consortium recommendations [1].

A cluster of families bearing *CTNNA1* germline truncating variants in the last exon and classified as variants of unknown significance (VUS) did not present HDGC-associated phenotypes. Individuals from these families presented only BC and DBC, which hints toward an association of variants located after the NMD recognition boundary and different types of cancer. Further research is required to establish an NMD recognition boundary and better define *CTNNA1* variants more closely related to HDGC. This is expected to decrease the uncertainty related to the clinical management of patients bearing truncating variants in this region of the gene [57, 63].

5.5 *CTNNA1* Germline Missense Variants Predispose to MDPT2, But Not HDGC

CTNNA1 germline missense variants have been associated with macular dystrophy patterned-2 (MDPT2) and, more recently, familial exudative vitreoretinopathy (FEVR) [64, 65].

Fig. 5.3 (continued) benign or likely benign variants. Exons with a darker gray tone are out-of-frame exons while with a lighter gray tone represent in-frame exons. Each geometric figure represents a different molecular variant type and the respective number of geometric figures in each variant represents the number of families carrying that variant. Variants are represented with the respective nucleotide and amino acid change. aa—Amino acid; nc—nucleotide

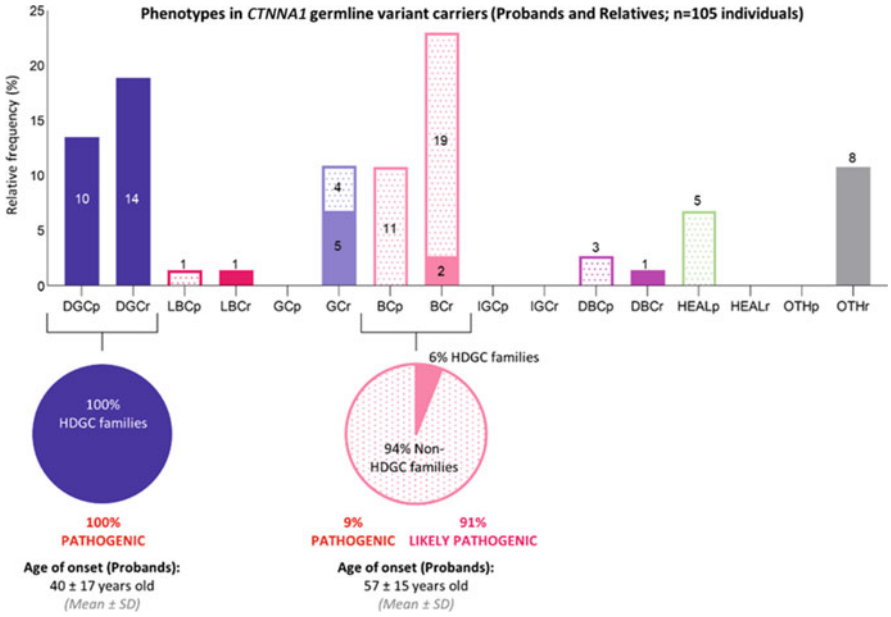


Fig. 5.4 Genotype-phenotype correlations in families carrying *CTNNA1* germline variants. Analysis of the phenotypes found in families carrying *CTNNA1* germline variants independently of the HDGC clinical criteria met. All phenotypes in the X axis with "p" means the phenotype encountered in probands while "r" means the phenotypes encountered in relatives. Full-colored bars represent families carrying a *CTNNA1* variant and that meet HDGC while dotted bars represent families carrying a *CTNNA1* variant and that do not meet HDGC criteria. BC—breast cancer of unspecified histotype; DBC—ductal breast cancer; DGC—diffuse gastric cancer; GC—gastric cancer; HEAL—healthy; IGC—intestinal gastric cancer; LBC—lobular breast cancer; OTH—others

MDPT2 is an autosomal dominant disease of the retinal pigment epithelium (RPE) which was first described by Deutman and Colleagues in 1970 [66] as a bilateral pigment accumulation in the macular area, resembling a butterfly wing patterning (also named butterfly-shaped pigmentary). From a certain age in adulthood, individuals with this disorder may present a slightly diminished best-corrected visual acuity and color vision [67, 68], which can ultimately progress to retina atrophy and underlying choroid in the macula [68, 69] and also to subretinal neovascularization [70], both resulting in severe vision loss.

FEVR is a severe inherited retinal disorder that is characterized by an incomplete vascularization of the peripheral retina and by the absence/abnormality of the secondary and tertiary capillary layers in the deep retina [71]. As a consequence, there is neovascularization and exudate, vitreous hemorrhaging, vitreous membranes' traction, macula displacement, and retina folding and detachment [72]. FEVR presents an incomplete penetrance and its clinical features are highly heterogeneous, being complete blindness the most severe disease phenotype [73].

In 2016, Saksens and Colleagues [64] reported three families with hereditary MDPT2. All had typical butterfly-shape pigment dystrophy and using whole exome sequencing, they identified three different heterozygous *CTNNA1* germline missense variants (*CTNNA1*: c.953 T > C; c.1293 T > G; and c.919G > A) (Fig. 5.3). All missense variants were located in very conserved protein regions. Notably, the authors created a mouse model carrying a coding missense homozygous mutation in the murine *CTNNA1* homolog gene [64]. This mouse line presented a similar disease phenotype in the RPE, with the *CTNNA1* variant co-segregating with disease for several generations. Moreover, the authors demonstrate that *CTNNA1* RNA and protein were still expressed in the RPE, which indicates that disease phenotypes may be a consequence of the perturbation of *CTNNA1* function, specifically in the RPE. In 2021, Tanner and Colleagues [74] identified six additional MDPT2 families carrying *CTNNA1* germline missense variants, thus confirming the association. Four distinct novel missense variants were identified and co-segregated with MDPT2 (Fig. 5.3). Interestingly, all four variants are located in the same region as the first three reported variants, which indicates that non-truncating changes in the vinculin binding and M-fragment domains may result specifically in macular dystrophy phenotypes.

A third study [65] identified heterozygous *CTNNA1* germline variants in three different families with FEVR out of 47 families studied. Contrarily to MDPT2, in this case, *CTNNA1* germline variants are scattered across the gene and only two are missense, being the other a frameshift variant (Fig. 5.3). Interestingly, the authors found that the frameshift variant caused the most severe phenotype among the three variants. The missense variant located in the β-catenin binding/homodimerization domain and the frameshift located in the vinculin binding domain completely lost the ability to bind to β-catenin, which consequently overactivated the expression of the Norrin/β-catenin signaling pathway and disrupted the AJC in cells.

5.6 Possible Mechanisms Underlying Tumor Formation and Development Due to *CTNNA1*/αE-catenin Impairment

The AJC is extremely important to maintain proper cell-cell adhesion, cellular polarity, and tissue organization. Impairment of the main proteins of the AJC is demonstrated to disrupt its normal function, resulting in initiation, development, and progression of several tumor types [28, 29, 75].

Besides the clear association of *CTNNA1*/αE-catenin impairment with development of early-onset DGC, *CTNNA1*/αE-catenin-specific single nucleotide missense alterations in conserved regions result in the development of MDPT2. Furthermore, it has been shown that αE-catenin is downregulated somatically in other types of cancer [9, 12, 14, 15, 22], such as lung [76, 77], breast [78–80], colon [81], and prostate cancer [82] and also myeloid leukemia [83, 84]. Below, we will present possible molecular mechanisms underlying the tumor formation and development associated with αE-catenin impairment.

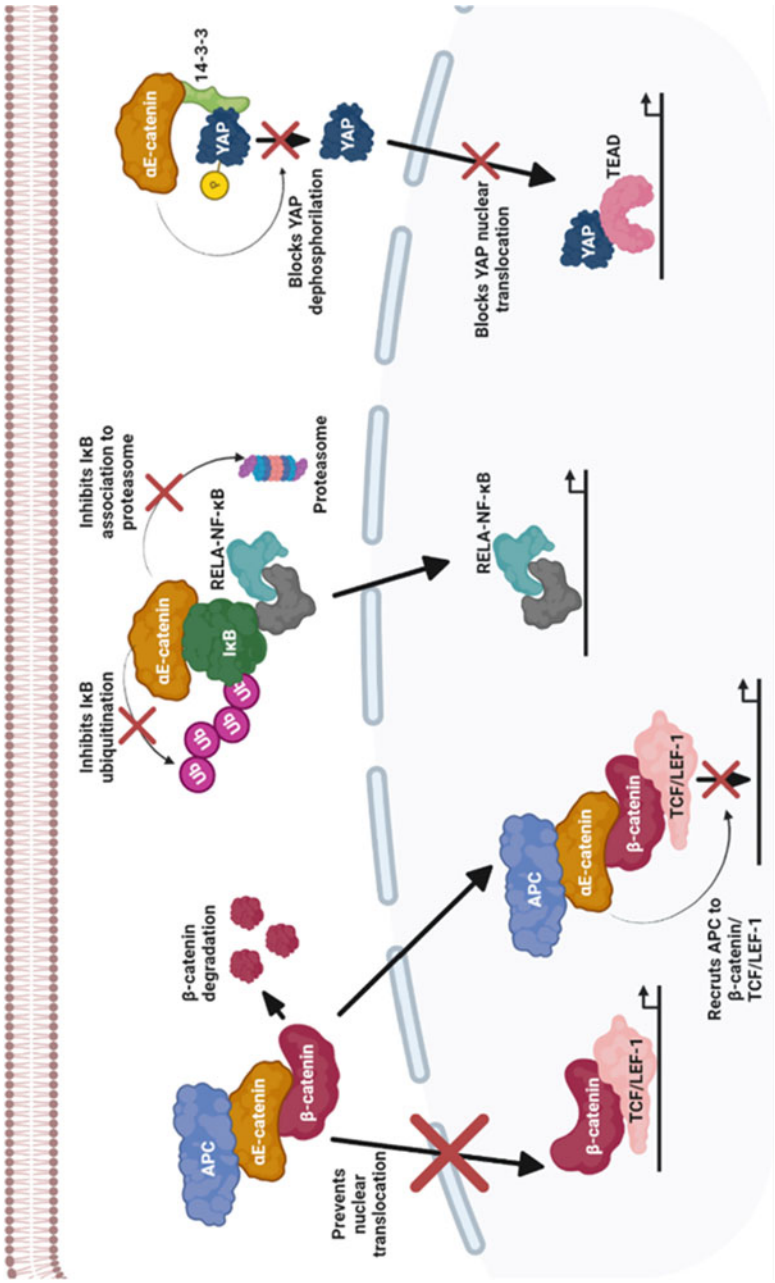


Fig. 5.5 Signaling pathways regulated by αE-catenin in cancer. αE-catenin maintains the integrity of the cadherin-catenin complex and binds the adherens junction complex to the actin cytoskeleton. Besides these functions, αE-catenin also regulates different signaling pathways that, upon dysregulation, are associated with cancer development. (Left side) αE-catenin is responsible for inhibiting the Wnt/β-catenin signaling pathway by: preventing formation of the

5.6.1 Wnt/β-catenin Signaling Pathway

The β-catenin protein links the AJC to the Wnt/Wingless signaling pathway. β-catenin stops being phosphorylated by glycogen synthase kinase-3 when Wnt ligands are present. This reduces degradation and stabilizes β-catenin which, in turn, enters the nucleus, binds to TCF/LEF-1 transcription factor and ultimately activates transcription of specific genes that are involved in cell proliferation, migration, and invasion [85] (Fig. 5.5). The activation of this signaling pathway is highly associated with tumor initiation and development in several different types of cancer [86–93].

It was demonstrated by Ji and colleagues [94] that ectopic expression of αE-catenin in glioma cells favored β-catenin retention in the cytoplasm and, upon αE-catenin knockdown by short hairpin RNA, β-catenin nuclear translocation is promoted. Furthermore, Choi and colleagues [95] demonstrated that αE-catenin interacts directly with the catenin inhibitory domain of the APC tumor suppressor gene. The stable binding of αE-catenin to APC promotes β-catenin ubiquitination and degradation. Also, the authors show that APC is translocated to the nucleus when interacting with αE-catenin, producing the APC:αE-catenin:β-catenin complex that interacts with CtBP:CoREST:LSD1 histone H3K4 demethylase and consequently inhibits transcription of Wnt target genes. Moreover, Giannini and colleagues [96] reported that αE-catenin is present at the nuclei of two colon cancer cell lines. Upon αE-catenin deficiency, cells displayed increased β-catenin-TCF transcriptional activity, which promotes transcription of Wnt target genes. This was repressed by αE-catenin ectopic expression fused to a nuclear localization signal.

Overall, the above studies demonstrate that α-catenin is capable of suppressing the Wnt/β-catenin by different mechanisms, such as β-catenin degradation, prevention of β-catenin translocation to the nucleus, or recruitment of APC to the β-catenin-TCF complex (Fig. 5.5). Nevertheless, further investigation is required to understand if β-catenin inhibition by α-catenin mediates a tumor-suppressing effect [9].

5.6.2 NF-κB Signaling Pathway

The nuclear factor κB signaling pathway (NF-κB) is extremely important in several cell mechanisms, such as survival, proliferation, angiogenesis, apoptosis, migration,

Fig. 5.5 (continued) β-catenin-TCF complex by inhibiting β-catenin nuclear translocation or by recruiting APC to the complex in the nucleus; promoting β-catenin degradation in the cytoplasm. (Middle) αE-catenin suppresses NF-κB signaling pathway by: inhibiting the ubiquitination of NF-κB inhibitor and inhibiting NF-κB inhibitor association to the proteasome. (Right side) αE-catenin regulates the Hippo-YAP signaling pathway by: blocking dephosphorylation of YAP and blocking YAP nuclear translocation

invasion, and immune response. Furthermore, hyperactivation of this signaling pathway is common in cancer development, being observed in both hematopoietic and solid tumors. The NF-κB family comprises five subunits that form both homo and heterodimers. In normal conditions, dimers are mainly located in the cytoplasm where they are associated with NF-κB inhibitor and, consequently, are transcriptionally inactive. Several cytokines, growth factors, and kinases can activate NF-κB through the canonical or non-canonical pathways [9, 97, 98].

A study conducted by Vasioukhin and colleagues [99] demonstrated that depletion of αE-catenin in mouse skin resulted in several skin and limb defects and also in death at an early age. A microarray profiling of αE-catenin-knockout mice showed a high number of NF-κB targeted genes being upregulated; nevertheless, the mechanism by which αE-catenin impairment upregulates these genes was not reported. Furthermore, Piao and colleagues [100] discovered that αE-catenin works as a tumor suppressor in basal-like breast cancer cases that do not express E-cadherin. This appears to be mediated by αE-catenin with NF-κB inhibitor which, in turn, upregulates the NF-κB signaling pathway specifically in this type of cancer. The authors used several breast cancer cell lines to deplete αE-catenin and further reconstituted its expression and discovered that α-catenin ectopic expression inhibited breast cancer cell proliferation, while α-catenin knockdown increased cell proliferation, cell migration and acquired anchorage-independent growth. Biochemical analysis on these cells showed that α-catenin physically interacts with and stabilizes NF-κB inhibitor by blocking its lysine 48-linked poly-ubiquitination and its association with the proteasome (Fig. 5.5). αE-catenin is downregulated in basal-like breast cancer and these results demonstrate the tumor-suppressing effect of αE-catenin in this type of tumor, mediated by NF-κB signaling pathway regulation.

5.6.3 Hippo-YAP Signaling Pathway

Hippo-YAP is a signaling pathway highly important to properly regulate organ size and its dysregulation results in tumor initiation and development [101, 102]. In particular, it has been shown that deregulation of the Hippo-YAP signaling pathway resulted in development of hepatocellular carcinoma in mice [103–108], in higher proliferation of colon cancer cells [109], and in higher tumorigenesis and metastasis capacity of breast cancer cells [110–113].

Silvis and colleagues [114] depleted α-catenin in stem and progenitor cells of hair follicles. This resulted in skin squamous cell carcinoma development in mice and, interestingly, the phenotype in α-catenin depleted mice was very similar to the one found in mice overexpressing YAP in the skin [115]. Furthermore, the authors show that proliferation of α-catenin-deficient cells is blocked when YAP is depleted by RNA interference. YAP sequestration increases due to α-catenin which, consequently, inhibits YAP transcriptional activity. The YAP regulation mediated by α-catenin does not involve the regulation of Hippo but, instead, is solely mediated by α-catenin and YAP interaction. The association of α-catenin with YAP is mediated by the 14-3-3 protein, which inhibits YAP dephosphorylation and

activation (Fig. 5.5) and, upon α-catenin depletion, skin tumors may develop due to increased YAP nuclear localization and transcriptional activity [114, 115]. Whether deregulation of the Hippo-YAP pathway by α-catenin depletion occurs in other types of cancer is yet to be determined.

5.6.4 Hedgehog Signaling Pathway

The Hedgehog signaling pathway was first identified in *Drosophila* as an important segmental patterning mediator during embryonic development and is highly conserved in vertebrates [116–118]. In mammals, the Hedgehog signaling pathway is especially important during embryonic development by regulating cell proliferation, migration, and differentiation and, afterward, is silenced in most adult tissues. Nevertheless, the central nervous system and the lungs rely on continuous Hedgehog signaling for tissue homeostasis and repair following injury [9, 119–122]. Deregulation of the Hedgehog signaling pathway has been demonstrated to promote development of several cancer types, such as lung, breast, brain, colon, and pancreatic cancer [119, 123].

Lien and colleagues [124] explored the function of α-catenin in the brain and discovered that αE-catenin is expressed by neural progenitors and αN-catenin by differentiated neurons. The authors depleted αE-catenin specifically in the brain of mice which resulted in very early death, with mice exhibiting enlarged heads with massive brain hyperplasia. Interestingly, a microarray analysis demonstrated that the most upregulated genes upon αE-catenin knockdown in the brain are two well-known target genes of the Hedgehog signaling pathway, hinting that αE-catenin loss in the central nervous system activates the Hedgehog signaling pathway. Nevertheless, further studies are required to understand by which mechanism this occurs.

5.7 Concluding Remarks

- *CTNNA1* germline variants, classified as PV, predispose to early-onset diffuse gastric cancer.
- *CTNNA1* germline variants, classified as LPV, do not predispose to diffuse gastric cancer and are associated with breast cancer of unknown histotype.
- There is still insufficient data supporting an association between *CTNNA1* germline variants and lobular breast cancer.
- *CTNNA1* germline missense variants predispose to macular dystrophy patterned 2, but not to hereditary diffuse gastric cancer.
- αE-catenin is responsible for connecting the adherens junction complex to the actin cytoskeleton.
- αE-catenin interacts with several proteins and regulates four different signaling pathways highly associated with cancer development.

- Impairment of *CTNNA1* leads to the development of multiple types of sporadic cancer, possibly due to its important role in the adherens junction complex, the actin cytoskeleton and in several cancer-associated signaling pathways.

References

1. Blair VR, McLeod M, Carneiro F, Coit DG, D'Addario JL, van Dieren JM et al (2020) Hereditary diffuse gastric cancer: updated clinical practice guidelines. Lancet Oncol 21(8): e386–ee97. https://doi.org/10.1016/S1470-2045(20)30219-9
2. Guilford P, Hopkins J, Harraway J, McLeod M, McLeod N, Harawira P et al (1998) E-cadherin germline mutations in familial gastric cancer. Nature 392(6674):402–405. https://doi.org/10.1038/32918
3. Oliveira C, Senz J, Kaurah P, Pinheiro H, Sanges R, Haegert A et al (2009) Germline CDH1 deletions in hereditary diffuse gastric cancer families. Hum Mol Genet 18(9):1545–1555. https://doi.org/10.1093/hmg/ddp046
4. Lobo S, Benusiglio PR, Coulet F, Boussemart L, Golmard L, Spier I et al (2021) Cancer predisposition and germline CTNNA1 variants. Eur J Med Genet 64(10):104316. https://doi.org/10.1016/j.ejmg.2021.104316
5. Majewski IJ, Kluijt I, Cats A, Scerri TS, de Jong D, Kluin RJ et al (2013) An α-E-catenin (CTNNA1) mutation in hereditary diffuse gastric cancer. J Pathol 229(4):621–629. https://doi.org/10.1002/path.4152
6. Dufour S, Mège R-M, Thiery JP (2013) α-catenin, vinculin, and F-actin in strengthening E-cadherin cell-cell adhesions and mechanosensing. Cell Adhes Migr 7(4):345–350. https://doi.org/10.4161/cam.25139
7. Takeichi M (2018) Multiple functions of α-catenin beyond cell adhesion regulation. Curr Opin Cell Biol 54:24–29. https://doi.org/10.1016/j.ceb.2018.02.014
8. Maiden SL, Hardin J (2011) The secret life of α-catenin: moonlighting in morphogenesis. J Cell Biol 195(4):543–552. https://doi.org/10.1083/jcb.201103106
9. Sun Y, Zhang J, Ma L, α-catenin. (2014) A tumor suppressor beyond adherens junctions. Cell cycle (Georgetown, Tex). 13(15):2334–2339. https://doi.org/10.4161/cc.29765
10. Choi HJ, Pokutta S, Cadwell GW, Bobkov AA, Bankston LA, Liddington RC et al (2012) αE-catenin is an autoinhibited molecule that coactivates vinculin. Proc Natl Acad Sci U S A 109(22):8576–8581. https://doi.org/10.1073/pnas.1203906109
11. McCrea PD, Gottardi CJ (2016) Beyond beta-catenin: prospects for a larger catenin network in the nucleus. Nat Rev Mol Cell Biol 17(1):55–64. https://doi.org/10.1038/nrm.2015.3
12. Vite A, Li J, Radice GL (2015) New functions for alpha-catenins in health and disease: from cancer to heart regeneration. Cell Tissue Res 360(3):773–783. https://doi.org/10.1007/s00441-015-2123-x
13. Biswas KH (2018) Regulation of α-catenin conformation at cadherin adhesions. J Biomech Sci Eng 13(4):17-00699-17. https://doi.org/10.1299/jbse.17-00699
14. Kobielak A, Fuchs E (2004) Alpha-catenin: at the junction of intercellular adhesion and actin dynamics. Nat Rev Mol Cell Biol 5(8):614–625. https://doi.org/10.1038/nrm1433
15. Benjamin JM, Nelson WJ (2008) Bench to bedside and back again: molecular mechanisms of alpha-catenin function and roles in tumorigenesis. Semin Cancer Biol 18(1):53–64. https://doi.org/10.1016/j.semcancer.2007.08.003
16. Vestweber D, Kemler R (1984) Rabbit antiserum against a purified surface glycoprotein decompacts mouse preimplantation embryos and reacts with specific adult tissues. Exp Cell Res 152(1):169–178. https://doi.org/10.1016/0014-4827(84)90241-6
17. Vestweber D, Kemler R (1984) Some structural and functional aspects of the cell adhesion molecule uvomorulin. Cell Differ 15(2–4):269–273. https://doi.org/10.1016/0045-6039(84)90084-8

18. Bays JL, DeMali KA (2017) Vinculin in cell-cell and cell-matrix adhesions. Cellular Mol Life Sci 74(16):2999–3009. https://doi.org/10.1007/s00018-017-2511-3
19. Goldmann WH (2016) Role of vinculin in cellular mechanotransduction. Cell Biol Int 40(3): 241–256. https://doi.org/10.1002/cbin.10563
20. Leerberg JM, Yap AS (2013) Vinculin, cadherin mechanotransduction and homeostasis of cell-cell junctions. Protoplasma 250(4):817–829. https://doi.org/10.1007/s00709-012-0475-6
21. Desai R, Sarpal R, Ishiyama N, Pellikka M, Ikura M, Tepass U (2013) Monomeric α-catenin links cadherin to the actin cytoskeleton. Nat Cell Biol 15(3):261–273. https://doi.org/10.1038/ncb2685
22. Gloushankova NA, Rubtsova SN, Zhitnyak IY (2017) Cadherin-mediated cell-cell interactions in normal and cancer cells. Tissue Barriers 5(3):e1356900. https://doi.org/10.1080/21688370.2017.1356900
23. Yamada S, Pokutta S, Drees F, Weis WI, Nelson WJ (2005) Deconstructing the cadherin-catenin-actin complex. Cell 123(5):889–901. https://doi.org/10.1016/j.cell.2005.09.020
24. Brüser L, Bogdan S (2017) Adherens junctions on the move-membrane trafficking of E-cadherin. Cold Spring Harb Perspect Biol 9(3). https://doi.org/10.1101/cshperspect.a029140
25. Mège RM, Ishiyama N (2017) Integration of cadherin adhesion and cytoskeleton at Adherens junctions. Cold Spring Harb Perspect Biol 9(5). https://doi.org/10.1101/cshperspect.a028738
26. Yonemura S (2017) Actin filament association at adherens junctions. J Med Investig 64(1.2): 14–19. https://doi.org/10.2152/jmi.64.14
27. Nelson WJ (2008) Regulation of cell-cell adhesion by the cadherin-catenin complex. Biochem Soc Trans 36(Pt 2):149–155. https://doi.org/10.1042/bst0360149
28. Garcia MA, Nelson WJ, Chavez N (2018) Cell-cell junctions organize structural and signaling networks. Cold Spring Harb Perspect Biol 10(4). https://doi.org/10.1101/cshperspect.a029181
29. Kaszak I, Witkowska-Piłaszewicz O, Niewiadomska Z, Dworecka-Kaszak B, Ngosa Toka F, Jurka P (2020) Role of Cadherins in cancer-a review. Int J Mol Sci 21(20). https://doi.org/10.3390/ijms21207624
30. Huber AH, Stewart DB, Laurents DV, Nelson WJ, Weis WI (2001) The cadherin cytoplasmic domain is unstructured in the absence of beta-catenin. A possible mechanism for regulating cadherin turnover. J Biol Chem 276(15):12301–12309. https://doi.org/10.1074/jbc.M010377200
31. Hinck L, Näthke IS, Papkoff J, Nelson WJ (1994) Dynamics of cadherin/catenin complex formation: novel protein interactions and pathways of complex assembly. J Cell Biol 125(6): 1327–1340. https://doi.org/10.1083/jcb.125.6.1327
32. Aberle H, Butz S, Stappert J, Weissig H, Kemler R, Hoschuetzky H (1994) Assembly of the cadherin-catenin complex in vitro with recombinant proteins. J Cell Sci 107(Pt 12): 3655–3663. https://doi.org/10.1242/jcs.107.12.3655
33. Davis MA, Ireton RC, Reynolds AB (2003) A core function for p120-catenin in cadherin turnover. J Cell Biol 163(3):525–534. https://doi.org/10.1083/jcb.200307111
34. Sept D, Elcock AH, McCammon JA (1999) Computer simulations of actin polymerization can explain the barbed-pointed end asymmetry1 1Edited by B. Honig. J Mol Biol 294(5): 1181–1189. https://doi.org/10.1006/jmbi.1999.3332
35. Skau CT, Waterman CM (2015) Specification of architecture and function of actin structures by actin nucleation factors. Annu Rev Biophys 44:285–310. https://doi.org/10.1146/annurev-biophys-060414-034308
36. Tobacman LS, Korn ED (1983) The kinetics of actin nucleation and polymerization. J Biol Chem 258(5):3207–3214
37. Cronin NM, DeMali KA (2022) Dynamics of the actin cytoskeleton at adhesion complexes. Biology 11(1). https://doi.org/10.3390/biology11010052
38. Blanchoin L, Boujemaa-Paterski R, Sykes C, Plastino J (2014) Actin dynamics, architecture, and mechanics in cell motility. Physiol Rev 94(1):235–263. https://doi.org/10.1152/physrev.00018.2013

39. Adhikari S, Moran J, Weddle C, Hinczewski M (2018) Unraveling the mechanism of the cadherin-catenin-actin catch bond. PLoS Comput Biol 14(8):e1006399. https://doi.org/10.1371/journal.pcbi.1006399
40. Borghi N, Sorokina M, Shcherbakova OG, Weis WI, Pruitt BL, Nelson WJ et al (2012) E-cadherin is under constitutive actomyosin-generated tension that is increased at cell-cell contacts upon externally applied stretch. Proc Natl Acad Sci U S A 109(31):12568–12573. https://doi.org/10.1073/pnas.1204390109
41. Pinheiro D, Bellaïche Y (2018) Mechanical force-driven adherens junction remodeling and epithelial dynamics. Dev Cell 47(1):3–19. https://doi.org/10.1016/j.devcel.2018.09.014
42. Drees F, Pokutta S, Yamada S, Nelson WJ, Weis WI (2005) Alpha-catenin is a molecular switch that binds E-cadherin-beta-catenin and regulates actin-filament assembly. Cell 123(5):903–915. https://doi.org/10.1016/j.cell.2005.09.021
43. Rimm DL, Koslov ER, Kebriaei P, Cianci CD, Morrow JS (1995) Alpha 1(E)-catenin is an actin-binding and -bundling protein mediating the attachment of F-actin to the membrane adhesion complex. Proc Natl Acad Sci U S A 92(19):8813–8817. https://doi.org/10.1073/pnas.92.19.8813
44. Bianchini JM, Kitt KN, Gloerich M, Pokutta S, Weis WI, Nelson WJ (2015) Reevaluating αE-catenin monomer and homodimer functions by characterizing E-cadherin/αE-catenin chimeras. J Cell Biol 210(7):1065–1074. https://doi.org/10.1083/jcb.201411080
45. Abe K, Takeichi M (2008) EPLIN mediates linkage of the cadherin catenin complex to F-actin and stabilizes the circumferential actin belt. Proc Natl Acad Sci U S A 105(1):13–19. https://doi.org/10.1073/pnas.0710504105
46. Hansford S, Kaurah P, Li-Chang H, Woo M, Senz J, Pinheiro H et al (2015) Hereditary diffuse gastric cancer syndrome: CDH1 mutations and beyond. JAMA Oncol 1(1):23–32. https://doi.org/10.1001/jamaoncol.2014.168
47. Pharoah PD, Guilford P, Caldas C (2001) Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. Gastroenterology 121(6):1348–1353. https://doi.org/10.1053/gast.2001.29611
48. Roberts ME, Ranola JMO, Marshall ML, Susswein LR, Graceffo S, Bohnert K et al (2019) Comparison of CDH1 penetrance estimates in clinically ascertained families vs families ascertained for multiple gastric cancers. JAMA Oncol 5(9):1325–1331. https://doi.org/10.1001/jamaoncol.2019.1208
49. Xicola RM, Li S, Rodriguez N, Reinecke P, Karam R, Speare V et al (2019) Clinical features and cancer risk in families with pathogenic CDH1 variants irrespective of clinical criteria. J Med Genet 56(12):838–843. https://doi.org/10.1136/jmedgenet-2019-105991
50. Caldas C, Carneiro F, Lynch HT, Yokota J, Wiesner GL, Powell SM et al (1999) Familial gastric cancer: overview and guidelines for management. J Med Genet 36(12):873–880
51. Fitzgerald RC, Hardwick R, Huntsman D, Carneiro F, Guilford P, Blair V et al (2010) Hereditary diffuse gastric cancer: updated consensus guidelines for clinical management and directions for future research. J Med Genet 47(7):436–444. https://doi.org/10.1136/jmg.2009.074237
52. van der Post RS, Vogelaar IP, Carneiro F, Guilford P, Huntsman D, Hoogerbrugge N et al (2015) Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. J Med Genet 52(6):361–374. https://doi.org/10.1136/jmedgenet-2015-103094
53. Weren RDA, van der Post RS, Vogelaar IP, van Krieken JH, Spruijt L, Lubinski J et al (2018) Role of germline aberrations affecting CTNNA1, MAP3K6 and MYD88 in gastric cancer susceptibility. J Med Genet 55(10):669–674. https://doi.org/10.1136/jmedgenet-2017-104962
54. Fewings E, Larionov A, Redman J, Goldgraben MA, Scarth J, Richardson S et al (2018) Germline pathogenic variants in PALB2 and other cancer-predisposing genes in families with hereditary diffuse gastric cancer without CDH1 mutation: a whole-exome sequencing study. Lancet Gastroenterol Hepatol 3(7):489–498. https://doi.org/10.1016/s2468-1253(18)30079-7

55. Gaston D, Hansford S, Oliveira C, Nightingale M, Pinheiro H, Macgillivray C et al (2014) Germline mutations in MAP3K6 are associated with familial gastric cancer. PLoS Genet 10(10):e1004669. https://doi.org/10.1371/journal.pgen.1004669
56. Benusiglio PR, Colas C, Guillerm E, Canard A, Delhomelle H, Warcoin M et al (2019) Clinical implications of CTNNA1 germline mutations in asymptomatic carriers. Gastric Cancer 22(4):899–903. https://doi.org/10.1007/s10120-018-00907-7
57. Lee K, Krempely K, Roberts ME, Anderson MJ, Carneiro F, Chao E et al (2018) Specifications of the ACMG/AMP variant curation guidelines for the analysis of germline CDH1 sequence variants. Hum Mutat 39(11):1553–1568. https://doi.org/10.1002/humu.23650
58. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17(5):405–423. https://doi.org/10.1038/gim.2015.30
59. Clark DF, Michalski ST, Tondon R, Nehoray B, Ebrahimzadeh J, Hughes SK et al (2020) Loss-of-function variants in CTNNA1 detected on multigene panel testing in individuals with gastric or breast cancer. Genet Med 22(5):840–846. https://doi.org/10.1038/s41436-020-0753-1
60. Shirts BH, Casadei S, Jacobson AL, Lee MK, Gulsuner S, Bennett RL et al (2016) Improving performance of multigene panels for genomic analysis of cancer predisposition. Genet Med 18(10):974–981. https://doi.org/10.1038/gim.2015.212
61. Khajavi M, Inoue K, Lupski JR (2006) Nonsense-mediated mRNA decay modulates clinical outcome of genetic disease. Eur J Hum Genet 14(10):1074–1081. https://doi.org/10.1038/sj.ejhg.5201649
62. Karam R, Carvalho J, Bruno I, Graziadio C, Senz J, Huntsman D et al (2008) The NMD mRNA surveillance pathway downregulates aberrant E-cadherin transcripts in gastric cancer cells and in CDH1 mutation carriers. Oncogene 27(30):4255–4260. https://doi.org/10.1038/onc.2008.62
63. Krempely K, Karam R (2018) A novel de novo CDH1 germline variant aids in the classification of carboxy-terminal E-cadherin alterations predicted to escape nonsense-mediated mRNA decay. Cold Spring Harbor Mol Case Stud 4(4):a003012. https://doi.org/10.1101/mcs.a003012
64. Saksens NTM, Krebs MP, Schoenmaker-Koller FE, Hicks W, Yu M, Shi L et al (2016) Mutations in CTNNA1 cause butterfly-shaped pigment dystrophy and perturbed retinal pigment epithelium integrity. Nat Genet 48(2):144–151. https://doi.org/10.1038/ng.3474
65. Zhu X, Yang M, Zhao P, Li S, Zhang L, Huang L et al (2021) Catenin α 1 mutations cause familial exudative vitreoretinopathy by overactivating Norrin/β-catenin signaling. J Clin Invest 131(6). https://doi.org/10.1172/jci139869
66. Deutman AF, van Blommestein JD, Henkes HE, Waardenburg PJ, Solleveld-van DE (1970) Butterfly-shaped pigment dystrophy of the fovea. Arch Ophthalmol (Chicago, Ill : 1960) 83(5):558–569. https://doi.org/10.1001/archopht.1970.00990030558006
67. Pinckers A (1988) Patterned dystrophies of the retinal pigment epithelium: a review. Ophthalmic Paediatr Genet 9(2):77–114. https://doi.org/10.3109/13816818809031483
68. Prensky JG, Bresnick GH (1983) Butterfly-shaped macular dystrophy in four generations. Arch Ophthalmol (Chicago, IL: 1960) 101(8):1198–1203. https://doi.org/10.1001/archopht.1983.01040020200005
69. van Lith-Verhoeven JJ, Cremers FP, van den Helm B, Hoyng CB, Deutman AF (2003) Genetic heterogeneity of butterfly-shaped pigment dystrophy of the fovea. Mol Vis 9:138–143
70. Marano F, Deutman AF, Aandekerk AL (1996) Butterfly-shaped pigment dystrophy of the fovea associated with subretinal neovascularization. Graefe's Arch Clin Exp Ophthalmol 234(4):270–274. https://doi.org/10.1007/bf00430421
71. Criswick VG, Schepens CL (1969) Familial exudative vitreoretinopathy. Am J Ophthalmol 68(4):578–594. https://doi.org/10.1016/0002-9394(69)91237-9

72. Collin Rob WJ, Nikopoulos K, Dona M, Gilissen C, Hoischen A, Boonstra FN et al (2013) ZNF408 is mutated in familial exudative vitreoretinopathy and is crucial for the development of zebrafish retinal vasculature. Proc Natl Acad Sci 110(24):9856–9861. https://doi.org/10.1073/pnas.1220864110
73. Robitaille J, MacDonald MLE, Kaykas A, Sheldahl LC, Zeisler J, Dubé M-P et al (2002) Mutant frizzled-4 disrupts retinal angiogenesis in familial exudative vitreoretinopathy. Nat Genet 32(2):326–330. https://doi.org/10.1038/ng957
74. Tanner A, Chan HW, Pulido JS, Arno G, Ba-Abbad R, Jurkute N et al (2021) Clinical and genetic findings in CTNNA1-associated macular pattern dystrophy. Ophthalmology 128(6):952–955. https://doi.org/10.1016/j.ophtha.2020.10.032
75. Vasioukhin V (2012) Adherens junctions and cancer. Subcell Biochem 60:379–414. https://doi.org/10.1007/978-94-007-4186-7_16
76. Shimoyama Y, Nagafuchi A, Fujita S, Gotoh M, Takeichi M, Tsukita S et al (1992) Cadherin dysfunction in a human cancer cell line: possible involvement of loss of alpha-catenin expression in reduced cell-cell adhesiveness. Cancer Res 52(20):5770–5774
77. Kimura K, Endo Y, Yonemura Y, Heizmann CW, Schafer BW, Watanabe Y et al (2000) Clinical significance of S100A4 and E-cadherin-related adhesion molecules in non-small cell lung cancer. Int J Oncol 16(6):1125–1131. https://doi.org/10.3892/ijo.16.6.1125
78. Hollestelle A, Elstrodt F, Timmermans M, Sieuwerts AM, Klijn JG, Foekens JA et al (2010) Four human breast cancer cell lines with biallelic inactivating alpha-catenin gene mutations. Breast Cancer Res Treat 122(1):125–133. https://doi.org/10.1007/s10549-009-0545-4
79. Craig DW, O'Shaughnessy JA, Kiefer JA, Aldrich J, Sinari S, Moses TM et al (2013) Genome and transcriptome sequencing in prospective metastatic triple-negative breast cancer uncovers therapeutic vulnerabilities. Mol Cancer Ther 12(1):104–116. https://doi.org/10.1158/1535-7163.Mct-12-0781
80. Ding L, Ellis MJ, Li S, Larson DE, Chen K, Wallis JW et al (2010) Genome remodelling in a basal-like breast cancer metastasis and xenograft. Nature 464(7291):999–1005. https://doi.org/10.1038/nature08989
81. Raftopoulos I, Davaris P, Karatzas G, Karayannacos P, Kouraklis G (1998) Level of alpha-catenin expression in colorectal cancer correlates with invasiveness, metastatic potential, and survival. J Surg Oncol 68(2):92–99. https://doi.org/10.1002/(sici)1096-9098(199806)68:2<92::aid-jso4>3.0.co;2-f
82. Aaltomaa S, Kärjä V, Lipponen P, Isotalo T, Kankkunen JP, Talja M et al (2005) Reduced alpha- and beta-catenin expression predicts shortened survival in local prostate cancer. Anticancer Res 25(6C):4707–4712
83. Horrigan SK, Arbieva ZH, Xie HY, Kravarusic J, Fulton NC, Naik H et al (2000) Delineation of a minimal interval and identification of 9 candidates for a tumor suppressor gene in malignant myeloid disorders on 5q31. Blood 95(7):2372–2377
84. Ye Y, McDevitt MA, Guo M, Zhang W, Galm O, Gore SD et al (2009) Progressive chromatin repression and promoter methylation of CTNNA1 associated with advanced myeloid malignancies. Cancer Res 69(21):8482–8490. https://doi.org/10.1158/0008-5472.CAN-09-1153
85. Huang H, He X (2008) Wnt/beta-catenin signaling: new (and old) players and new insights. Curr Opin Cell Biol 20(2):119–125. https://doi.org/10.1016/j.ceb.2008.01.009
86. Wang Y, van der Zee M, Fodde R, Blok LJ (2010) Wnt/B-catenin and sex hormone signaling in endometrial homeostasis and cancer. Oncotarget 1(7):674–684. https://doi.org/10.18632/oncotarget.101007
87. Chan DW, Mak CS, Leung TH, Chan KK, Ngan HY (2012) Down-regulation of Sox7 is associated with aberrant activation of Wnt/b-catenin signaling in endometrial cancer. Oncotarget 3(12):1546–1556. https://doi.org/10.18632/oncotarget.667
88. Pacheco-Pinedo EC, Morrisey EE (2011) Wnt and Kras signaling-dark siblings in lung cancer. Oncotarget 2(7):569–574. https://doi.org/10.18632/oncotarget.305

89. Allen JE, El-Deiry WS (2013) Calcein-effluxing human colon cancer cells are enriched for self-renewal capacity and depend on β-catenin. Oncotarget 4(2):184–191. https://doi.org/10.18632/oncotarget.883
90. Jaitner S, Reiche JA, Schäffauer AJ, Hiendlmeyer E, Herbst H, Brabletz T et al (2012) Human telomerase reverse transcriptase (hTERT) is a target gene of β-catenin in human colorectal tumors. Cell Cycle (Georgetown, Tex). 11(17):3331–3338. https://doi.org/10.4161/cc.21790
91. Reya T, Clevers H (2005) Wnt signalling in stem cells and cancer. Nature 434(7035):843–850. https://doi.org/10.1038/nature03319
92. Cha YH, Kim NH, Park C, Lee I, Kim HS, Yook JI (2012) MiRNA-34 intrinsically links p53 tumor suppressor and Wnt signaling. Cell Cycle (Georgetown, Tex) 11(7):1273–1281. https://doi.org/10.4161/cc.19618
93. Wang X, Meng X, Sun X, Liu M, Gao S, Zhao J et al (2009) Wnt/beta-catenin signaling pathway may regulate cell cycle and expression of cyclin a and cyclin E protein in hepatocellular carcinoma cells. Cell Cycle (Georgetown, Tex). 8(10):1567–1570. https://doi.org/10.4161/cc.8.10.8489
94. Ji H, Wang J, Fang B, Fang X, Lu Z (2011) α-Catenin inhibits glioma cell migration, invasion, and proliferation by suppression of β-catenin transactivation. J Neuro-Oncol 103(3):445–451. https://doi.org/10.1007/s11060-010-0413-4
95. Choi SH, Estarás C, Moresco JJ, Yates JR 3rd, Jones KA (2013) α-Catenin interacts with APC to regulate β-catenin proteolysis and transcriptional repression of Wnt target genes. Genes Dev 27(22):2473–2488. https://doi.org/10.1101/gad.229062.113
96. Giannini AL, Vivanco M, Kypta RM (2000) alpha-catenin inhibits beta-catenin signaling by preventing formation of a beta-catenin*T-cell factor*DNA complex. J Biol Chem 275(29):21883–21888. https://doi.org/10.1074/jbc.M001929200
97. Bassères DS, Baldwin AS (2006) Nuclear factor-kappaB and inhibitor of kappaB kinase pathways in oncogenic initiation and progression. Oncogene 25(51):6817–6830. https://doi.org/10.1038/sj.onc.1209942
98. Baud V, Karin M (2009) Is NF-kappaB a good target for cancer therapy? Hopes and pitfalls. Nat Rev Drug Discov 8(1):33–40. https://doi.org/10.1038/nrd2781
99. Vasioukhin V, Bauer C, Degenstein L, Wise B, Fuchs E (2001) Hyperproliferation and defects in epithelial polarity upon conditional ablation of alpha-catenin in skin. Cell 104(4):605–617. https://doi.org/10.1016/s0092-8674(01)00246-x
100. Piao HL, Yuan Y, Wang M, Sun Y, Liang H, Ma L (2014) α-catenin acts as a tumour suppressor in E-cadherin-negative basal-like breast cancer by inhibiting NF-κB signalling. Nat Cell Biol 16(3):245–254. https://doi.org/10.1038/ncb2909
101. Saucedo LJ, Edgar BA (2007) Filling out the Hippo pathway. Nat Rev Mol Cell Biol 8(8):613–621. https://doi.org/10.1038/nrm2221
102. Zhao B, Tumaneng K, Guan KL (2011) The Hippo pathway in organ size control, tissue regeneration and stem cell self-renewal. Nat Cell Biol 13(8):877–883. https://doi.org/10.1038/ncb2303
103. Camargo FD, Gokhale S, Johnnidis JB, Fu D, Bell GW, Jaenisch R et al (2007) YAP1 increases organ size and expands undifferentiated progenitor cells. Curr Biol 17(23):2054–2060. https://doi.org/10.1016/j.cub.2007.10.039
104. Dong J, Feldmann G, Huang J, Wu S, Zhang N, Comerford SA et al (2007) Elucidation of a universal size-control mechanism in Drosophila and mammals. Cell 130(6):1120–1133. https://doi.org/10.1016/j.cell.2007.07.019
105. Lee KP, Lee JH, Kim TS, Kim TH, Park HD, Byun JS et al (2010) The Hippo-Salvador pathway restrains hepatic oval cell proliferation, liver size, and liver tumorigenesis. Proc Natl Acad Sci U S A 107(18):8248–8253. https://doi.org/10.1073/pnas.0912203107
106. Lu L, Li Y, Kim SM, Bossuyt W, Liu P, Qiu Q et al (2010) Hippo signaling is a potent in vivo growth and tumor suppressor pathway in the mammalian liver. Proc Natl Acad Sci U S A 107(4):1437–1442. https://doi.org/10.1073/pnas.0911427107

107. Song H, Mak KK, Topol L, Yun K, Hu J, Garrett L et al (2010) Mammalian Mst1 and Mst2 kinases play essential roles in organ size control and tumor suppression. Proc Natl Acad Sci U S A 107(4):1431–1436. https://doi.org/10.1073/pnas.0911409107
108. Zhou D, Conrad C, Xia F, Park JS, Payer B, Yin Y et al (2009) Mst1 and Mst2 maintain hepatocyte quiescence and suppress hepatocellular carcinoma development through inactivation of the Yap1 oncogene. Cancer Cell 16(5):425–438. https://doi.org/10.1016/j.ccr.2009.09.026
109. Avruch J, Zhou D, Bardeesy N (2012) YAP oncogene overexpression supercharges colon cancer proliferation. Cell Cycle (Georgetown, Tex). 11(6):1090–1096. https://doi.org/10.4161/cc.11.6.19453
110. Chen Q, Zhang N, Gray RS, Li H, Ewald AJ, Zahnow CA et al (2014) A temporal requirement for Hippo signaling in mammary gland differentiation, growth, and tumorigenesis. Genes Dev 28(5):432–437. https://doi.org/10.1101/gad.233676.113
111. Chen D, Sun Y, Wei Y, Zhang P, Rezaeian AH, Teruya-Feldstein J et al (2012) LIFR is a breast cancer metastasis suppressor upstream of the Hippo-YAP pathway and a prognostic marker. Nat Med 18(10):1511–1517. https://doi.org/10.1038/nm.2940
112. Lamar JM, Stern P, Liu H, Schindler JW, Jiang ZG, Hynes RO (2012) The Hippo pathway target, YAP, promotes metastasis through its TEAD-interaction domain. Proc Natl Acad Sci U S A 109(37):E2441–E2450. https://doi.org/10.1073/pnas.1212021109
113. Piccolo S (2012) LIF-ting Hippo averts metastasis. Nat Med 18(10):1463–1465. https://doi.org/10.1038/nm.2955
114. Silvis MR, Kreger BT, Lien WH, Klezovitch O, Rudakova GM, Camargo FD et al (2011) α-catenin is a tumor suppressor that controls cell accumulation by regulating the localization and activity of the transcriptional coactivator Yap1. Sci Signal 4(174):ra33. https://doi.org/10.1126/scisignal.2001823
115. Schlegelmilch K, Mohseni M, Kirak O, Pruszak J, Rodriguez JR, Zhou D et al (2011) Yap1 acts downstream of α-catenin to control epidermal proliferation. Cell 144(5):782–795. https://doi.org/10.1016/j.cell.2011.02.031
116. Varjosalo M, Taipale J (2008) Hedgehog: functions and mechanisms. Genes Dev 22(18):2454–2472. https://doi.org/10.1101/gad.1693608
117. Ingham PW, McMahon AP (2001) Hedgehog signaling in animal development: paradigms and principles. Genes Dev 15(23):3059–3087. https://doi.org/10.1101/gad.938601
118. Nüsslein-Volhard C, Wieschaus E (1980) Mutations affecting segment number and polarity in Drosophila. Nature 287(5785):795–801. https://doi.org/10.1038/287795a0
119. Merchant AA, Matsui W (2010) Targeting Hedgehog--a cancer stem cell pathway. Clin Cancer Res 16(12):3130–3140. https://doi.org/10.1158/1078-0432.CCR-09-2846
120. Beachy PA, Karhadkar SS, Berman DM (2004) Tissue repair and stem cell renewal in carcinogenesis. Nature 432(7015):324–331. https://doi.org/10.1038/nature03100
121. Watkins DN, Berman DM, Burkholder SG, Wang B, Beachy PA, Baylin SB (2003) Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. Nature 422(6929):313–317. https://doi.org/10.1038/nature01493
122. Ahn S, Joyner AL (2005) In vivo analysis of quiescent adult neural stem cells responding to Sonic hedgehog. Nature 437(7060):894–897. https://doi.org/10.1038/nature03994
123. Ruat M, Hoch L, Faure H, Rognan D (2014) Targeting of smoothened for therapeutic gain. Trends Pharmacol Sci 35(5):237–246. https://doi.org/10.1016/j.tips.2014.03.002
124. Lien WH, Klezovitch O, Fernandez TE, Delrow J, Vasioukhin V (2006) alphaE-catenin controls cerebral cortical size by regulating the hedgehog signaling pathway. Science (New York, NY) 311(5767):1609–1612. https://doi.org/10.1126/science.1121449

Revisiting the Biological and Clinical Impact of *CDH1* Missense Variants

6

Joana Pereira, Soraia Melo, Patrícia Carneiro, Maria Sofia Fernandes, Joana Figueiredo, and Raquel Seruca

Abstract

E-cadherin, encoded by *CDH1*, plays an undisputable role in mechanical and biochemical signals that are crucial for cell integrity and tissue organization. Hence, E-cadherin deregulation results in severe tissue imbalances as those seen in cancer and congenital disorders. In particular, hereditary diffuse gastric cancer, lobular breast cancer, cleft lip/palate, and the blepharocheilodontic syndrome have been recognized as *CDH1*-associated entities. Among a plethora of *CDH1* genetic alterations identified in disease contexts, missense variants represent a huge burden for genetic counselling and patient management. Indeed, establishment of their biological and clinical impact is not always straightforward, contributing to misestimation and inaccurate classification. Herein, we provide an overview of the state of the art concerning *CDH1* missense variants, their geographical distribution and their relevance in distinct clinical spectra. We highlight the unequivocal value of an integrative pipeline to assess functional significance of variants, encompassing familial and population data analysis, in silico modelling, in vitro assays and in vivo studies. Importantly, we discuss how this strategy may improve genetic counselling of patients and their families,

J. Pereira · J. Figueiredo (✉) · R. Seruca
i3S – Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal

Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal

Medical Faculty of the University of Porto, Porto, Portugal
e-mail: jfigueiredo@ipatimup.pt

S. Melo · P. Carneiro · M. S. Fernandes
i3S – Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal

Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_6

whilst opening up avenues of research addressing the aetiology of E-cadherin-mediated disorders.

6.1 Introduction

Familial clustering of gastric cancer occurs in approximately 10% of cases, with 3% arising in the setting of hereditary diffuse gastric cancer (HDGC), an inherited cancer predisposition syndrome most often ascribed to *CDH1* inactivating germline mutations [1, 2]. More than 155 *CDH1* variants have been described in the context of HDGC, spanning the entire coding sequence and all protein functional domains [3]. Current guidelines recommend that, when diagnosed with a pathogenic germline variant, individuals should consider prophylactic total gastrectomy, which remains the mainstay of gastric cancer risk reduction [4]. Counselling of these individuals must balance the burden of gastric cancer risk and the psychological strain imposed by the dilemma of having to select between life-altering prophylactic surgery and intense endoscopic surveillance [4, 5].

Traditionally, detection of *CDH1* variants has been a consequence of the identification of individuals at risk for HDGC based on clinical criteria, which on itself has evolved significantly over the past 20 years [4, 6–8]. The emergence of multigene panel testing and advances in high-throughput sequencing technologies have uncovered a bulky amount of genetic variations, not only in the HDGC context but also in individuals without any personal or family history of diffuse gastric cancer. In fact, *CDH1* alterations are identified in a disease spectrum that comprises lobular breast cancer, colorectal cancer, cleft lip/palate, and blepharocheilodontic syndrome aside from diffuse-type gastric adenocarcinoma [9]. This unveils a serious challenge for clinical management of carriers and their families, given the difficulty of predicting variant phenotypic significance. In particular, in contrast to *CDH1* truncating mutations, which have a clear deleterious effect, mutations of the missense type require an extensive analysis that includes variant annotation, functional evaluation and validation in large sample sizes [10].

A critical issue surrounding *CDH1* missense variants is the incorporation of clinical information establishing their pathogenicity, which leads to misestimation of disease penetrance and, consequently, to inaccurate classification and propagation among populations. More so, screening programs vary with respect to the applied clinical criteria, as well as across health systems from diverse countries [11], contributing to absence of systematic results regarding *CDH1* screening and classification.

In this review, we highlight the current knowledge surrounding *CDH1* missense variants, focusing on their biological and clinical impact. Ultimately, we discuss future perspectives on variant classification guidelines, and whether these should cover disease-specific criteria and mutation geographic distribution.

6.2 *CDH1* Missense Variants in Distinct Clinical Phenotypes

The *CDH1* gene encodes E-cadherin, which is a calcium-dependent cell adhesion molecule essential for the maintenance of cell integrity and tissue organization [12]. E-cadherin has a unique ability to integrate cell-cell linkages, contractile forces, and biochemical signals regulating epithelial homeostasis. While its extracellular domain is responsible for homophilic binding of E-cadherin molecules on adjacent cells, the intracellular portion couples intercellular adhesion with the cytoskeleton [13]. At the biochemical level, this cytoskeletal network controls protein trafficking, modulating activity of Rho GTPases and growth factor receptors, as well as integrin delivery with impact in cell morphology and behaviour [14, 15]. Hence, it is not unforeseen that E-cadherin alterations result in cellular de-differentiation, invasion and tissue disorder.

Consistent with the complex role of E-cadherin in epithelia, *CDH1* germline variants have not only been associated with several cancer phenotypes, but also with severe congenital malformations [9]. Of note, there is no evidence supporting a genotype-phenotype correlation considering mutation type or localization, which impairs the establishment of precise management strategies and surveillance schemes.

Missense variants are reported in all clinical spectra, with the larger proportion identified in the HDGC setting ($n = 46$), possibly as a consequence of long-term research and focused patient screening. Lobular breast and colorectal cancers were subsequently associated with *CDH1* alterations, 9 and 4 of which, respectively, are of the missense-type (Table 6.1). In fact, missense mutations constitute the most frequent alteration in hereditary lobular breast cancer [16, 17]. Cleft lip/palate and the blepharocheilodontic syndrome were recently recognised as additional manifestations of *CDH1* loss and, to date, 15 and 7 missense variants were identified in these settings, respectively.

A striking observation is that variants affecting the same nucleotide, or even exactly the same missense variant, can be detected in patients displaying independent clinical entities. For instance, the *CDH1* c.88C > A (p.P30T) variant was described in HDGC, lobular breast cancer and cleft lip/palate [18–20]. Likewise, the *CDH1* c.2413G > A (p.D805N) was associated with HDGC and cleft lip/palate phenotypes [3, 20]. Ghoumid and colleagues state that *CDH1* missense variants yield similar effects in both blepharocheilodontic and HDGC syndromes [21], corroborating these observations. However, this issue has raised some controversy, namely by Kievit et al. who argue that the mutant protein in the blepharocheilodontic syndrome context dimerises with wild-type forms, exerting a dominant negative effect in the formation of adherent junctions [22].

Despite extensive research efforts, mechanisms underlying these pleiotropic outcomes remain to be identified. We envision that loss of E-cadherin induces an abnormal cell-extracellular matrix (ECM) interaction, implicating biomechanical imbalances and specific tissue rearrangements in the genesis of cancer development and morphogenetic defects. In this sense, we believe that the specificities of the ECM

Table 6.1 *CDH1* missense variants in different clinical phenotypes. For each missense variant described, the altered amino acid, exon, protein domain affected, clinical phenotype, as well as the corresponding reference are displayed. HGVS, human genome variation society nomenclature; EC, extracellular cadherin domain; HDGC, hereditary diffuse gastric cancer; DGC, diffuse gastric cancer; GC, gastric cancer; LBC, lobular breast cancer; BC, breast cancer; CRC, colorectal cancer; PC, prostate cancer; CL/P, cleft lip with or without cleft palate; BCDS, blepharocheilodontic syndrome

HGVS	Protein change	Exon	Domain	Clinical phenotype	References
2T > C	M1T	1	Signal peptide	HDGC	[74]
3G > A	M1I	1	Signal peptide	HDGC	[3]
3G > C	M1I	1	Signal peptide	HDGC	[75]
48G > C	Q16H	1	Signal peptide	HDGC	[37]
79C > T	P27S	2	Signal peptide	HDGC	[3]
88C > A	P30T	2	Precursor	HDGC	[18]
185G > T	G62V	3	Precursor	HDGC	[31, 76]
286A > G	I96V	3	Precursor	HDGC	[3]
313T > A	S105T	3	Precursor	HDGC	[77]
353C > G	T118R	3	Precursor	HDGC	[71, 78]
371G > A	R124H	3	Precursor	HDGC	[18]
387G > T	Q129H	3	Precursor	HDGC	[77]
515C > G	P172R	4	EC1	HDGC	[79]
554A > T	E185V	5	EC1	HDGC	[39]
635G > A	G212E	5	EC1	HDGC	[33]
641T > C	L214P	5	EC1	HDGC	[3, 31, 71]
670C > T	R224C	5	EC1	HDGC	[80, 81]
695C > G	S232C	6	EC1	HDGC	[39]
715G > A	G239R	6	EC1	HDGC	[3, 78, 82, 83]
731A > G	D244G	6	EC1	HDGC	[84]
808T > G	S270A	6	EC2	HDGC	[81, 85]
892G > A	A298T	7	EC2	HDGC	[3, 31, 86]
977T > A	I326N	7	EC2	HDGC	[87]
1018A > G	T340A	8	EC2	HDGC	[88]
1118C > T	P373L	8	EC2	HDGC	[89]
1130C > G	P377R	8	EC3	HDGC	[81]
1225T > C	W409R	9	EC3	HDGC	[86]
1243A > C	I415L	9	EC3	HDGC	[90]
1285C > T	P429S	9	EC3	HDGC	[75]
1460T > C	V487A	10	EC4	HDGC	[84]
1676G > A	S559N	11	EC4	HDGC	[77]
1679C > G	T560R	11	EC4	HDGC	[3]
1748T > G	L583R	12	EC4	HDGC	[57]

(continued)

Table 6.1 (continued)

HGVS	Protein change	Exon	Domain	Clinical phenotype	References
1774G > A	A592T	12	EC4	HDGC	[91]
1796C > G	T599S	12	EC5	HDGC	[82]
1806C > A	F602L	12	EC5	HDGC	[77]
1901C > T	A634V	12	EC5	HDGC	[55]
2195G > A	R732Q	14	Cytoplasmic	HDGC	[86]
2245C > T	R749W	14	Cytoplasmic	HDGC	[52, 82]
2248G > A	D750N	14	Cytoplasmic	HDGC	[3]
2269G > A	E757K	14	Cytoplasmic	HDGC	[52]
2315T > A	L772Q	15	Cytoplasmic	HDGC	[18]
2343A > T	E781D	15	Cytoplasmic	HDGC	[82]
2396C > G	P799R	15	Cytoplasmic	HDGC	[91]
2413G > A	D805N	15	Cytoplasmic	HDGC	[3]
2494G > A	V832M	16	Cytoplasmic	HDGC	[55]
1849G > A	A617T	12	EC5	DGC	[92]
2195G > A	R732Q	14	Cytoplasmic	DGC	[93]
604G > A	V202I	5	EC1	GC	[94]
820G > A	G274S	6	EC2	GC	[95]
1409C > T	T470I	10	EC3	GC	[96]
1679C > G	T560R	11	EC4	GC	[93]
8C > G	P3R	1	Signal peptide	LBC	[19]
88C > A	P30T	2	Precursor	LBC	[19, 97]
1223C > T	A408V	9	EC3	LBC	[19]
1297G > A	D433N	9	EC3	LBC	[19]
1679C > G	T560R	11	EC4	LBC	[93]
1813A > G	R605G	12	EC5	LBC	[19]
1876T > G	F626V	12	EC5	LBC	[97]
2195G > A	R732Q	14	Cytoplasmic	LBC	[93]
2494G > A	V832M	16	Cytoplasmic	LBC	[19]
715G > A	G239R	6	EC1	BC	[93]
1774G > A	A592T	12	EC4	BC	[19, 98]
1901C > T	A634V	12	EC5	BC	[93]
2512A > G	S838G	16	Cytoplasmic	BC	[98]
1018A > G	T340A	8	EC2	CRC	[99]
1225T > C	W409R	9	EC3	CRC	[86]
1774G > A	A592T	12	EC4	CRC	[100]
2195G > A	R732Q	14	Cytoplasmic	CRC	[93]
808T > G	S270A	6	EC2	PC	[85]
1774G > A	A592T	12	EC4	PC	[101]
2329G > A	D777N	15	Cytoplasmic	PC	[101]
1774G > A	A592T	12	EC4	Glioma	[102]
2450C > T	A817V	16	Cytoplasmic	Glioma	[102]

(continued)

Table 6.1 (continued)

HGVS	Protein change	Exon	Domain	Clinical phenotype	References
88C > A	P30T	2	Precursor	CL/P	[20]
337A > G	K113E	3	Precursor	CL/P	[20]
468G > C	W156C	4	EC1	CL/P	[103]
752C > T	T251M	6	EC1	CL/P	[104]
760G > A	D254N	6	EC1	CL/P	[104, 105]
768T > A	N256K	6	EC1	CL/P	[104]
1108G > T	D370Y	8	EC2	CL/P	[20]
1235T > C	V412A	9	EC3	CL/P	[106]
1273G > A	V425I	9	EC3	CL/P	[106]
1489G > A	E497K	10	EC4	CL/P	[104]
1565C > T	T522I	10	EC4	CL/P	[106]
1766A > T	N589I	12	EC4	CL/P	[104]
1888C > G	L630V	12	EC5	CL/P	[106]
2351G > A	R784H	15	Cytoplasmic	CL/P	[105]
2413G > A	D805N	15	Cytoplasmic	CL/P	[20]
760G > T	D254Y	6	EC1	BCDS	[21]
760G > A	D254N	6	EC1	BCDS	[22]
768T > G	N256K	6	EC1	BCDS	[22]
770A > T	D257V	6	EC1	BCDS	[21]
862G > C	D288H	7	EC2	BCDS	[22]
1118C > G	P373R	8	EC2	BCDS	[22]
2028C > A	D676E	13	EC5	BCDS	[107]

in each tissue context will be a determinant factor for the phenotypic manifestations of the same genotype.

6.3 Insights from Population Variation and Geographic Distribution of Variants

Ever since the identification of *CDH1* germline mutations in the Maori population, which dates back to 1998, a growing number of reports have documented a variety of *CDH1* alterations worldwide [2]. Indeed, *CDH1* alterations have been detected in different ethnic populations from distinct geographical parts of the globe [23], reflecting the variable screening programs and surveillance protocols, and ultimately gastric cancer incidence around the world [24]. Overall, a significantly higher gastric cancer frequency is found in developing regions such as Eastern Asia (China, Japan, Korea), Eastern Europe, and South America, whereas Africa, Northern America, Northern Europe, Australia, New Zealand, and South-Eastern Asia are regarded as low incidence areas [24]. Regardless of this general trend in gastric cancer incidence,

it is clear that sporadic and hereditary forms contrast in their geographical distribution.

E-cadherin plays an unequivocal role in HDGC, and *CDH1* germline alterations are causative events in about 40% of patients [6, 10, 25]. With the increasing reports of *CDH1* mutations, their worldwide distribution, type of mutation and screening settings in which they are identified are becoming more apparent [23, 26, 27]. Studies addressing incidence of *CDH1* germline mutations from low- and high-risk regions of gastric cancer have shown that E-cadherin genetic screenings performed in low-risk areas led to the identification of a higher frequency of *CDH1* germline mutations [11]. Concerning mutation type distribution, a recent study reported that deletions are more frequent in Europe, splice site in America, missense in Asia and non-sense in Oceania [23]. Missense mutations, in particular, represent 21% of mutations identified in Europe, 20% in America, and 68% in Asia, with a residual percentage in Oceania. The high incidence of missense mutations in Asia is mostly attributed to Korea and Japan, where extensive screening and early diagnosis programs are in place [28]. However, it is interesting that application of clinical criteria shifts the most frequently identified mutation types in all regions across the globe. Of note, missense mutations in this setting decreased in Asia (6%), possibly as a result of their low penetrance and consequent underestimation, and increased in Europe (33%), reflecting the awareness of missense clinical significance and dedicated research programs [23]. By establishing a comprehensive characterization score of missense variants, it was possible to observe that unbiased screening over selects individuals harbouring *CDH1* variants with an unclear association to gastric cancer risk. In contrast, the identification of individuals carrying missense mutations with clinical relevance is more likely to occur based upon family criteria [23].

This data demonstrates how effective screening programs, combining clinical criteria and accurate classification systems, can undoubtedly influence gastric cancer aetiology. More so, we highlight that demographic distribution of disease incidence cannot be explained by *CDH1* variants alone, but may also reflect the involvement of environmental factors and other genetic interactions.

6.4 Integrative Pipeline to Determine Variant Functional Significance

Irrespective of recent advances, an accurate prediction on whether or not variants are responsible for disorders is far from established. Further, most of *CDH1* rule specifications for the ACMG/AMP variant curation guidelines are not recommended for missense changes and a large proportion of variants remains unclassified [29].

To address the challenges in the management and surveillance of carriers and their families following the identification of missense variants, a complementary set of analysis encompassing familial and population data, in silico models, in vitro assays and in vivo studies can be implemented (Fig. 6.1) [10, 30]. We believe that variant pathogenicity cannot be determined based on standalone evidence from a single approach since all methods have limitations.

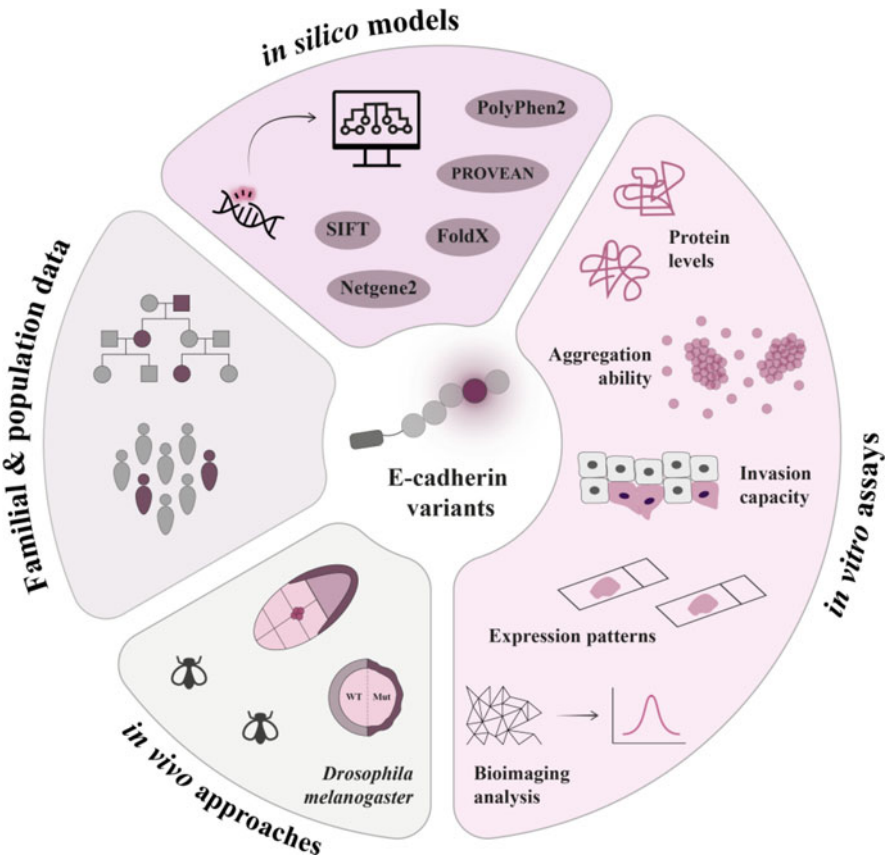


Fig. 6.1 Pipeline to address the significance of *CDH1* missense variants. A comprehensive analysis of family pedigree, taking into account the diverse clinical presentations of *CDH1* mutations, as well as variant frequencies across different ethnic groups can provide crucial information on possible causative effects. In silico models estimate the degree of conservation of mutated amino acids within species, their impact on splicing and on protein structure. In contrast, in vitro assays can be explored to investigate the impact of *CDH1* variants at the cellular level, namely on protein expression and localization, cell-cell aggregation ability, anti-invasive capacity, and intercellular organization. *Drosophila melanogaster* emerged as a valuable tool to evaluate cell migration dynamics and detect abnormal epithelial features in a physiological environment

6.4.1 Familial and Population Data

Familial and population data provide critical information for evaluation of *CDH1* variant effects and should be applied as a first-line approach. This includes analysis of mutation co-segregation with the disease within pedigrees, mutation recurrence in unrelated families, and mutation frequency in healthy control populations [31, 32].

The comprehensive examination of a family pedigree, taking into account diverse clinical presentations of *CDH1* mutations, may highlight a causative role for missense variants and predict disease risk in germline carriers [33]. Still, these studies can be demanding given the small size of the families and lack of information from patient relatives [31, 32]. The identification of recurrent variants segregating with gastric and breast cancer in unrelated families is also a strong indication of an associated deleterious effect [34]. Therefore, genetic counsellors must be constantly updated with respect to databases that assemble genetic conditions and familial history across clinical laboratories.

Variant frequencies have been used as an argument for classification according to the rare allele model, in which the evolutionary theory predicts that disease alleles should not be common. However, this model is not consistent with simulations of allele frequency distribution of data retrieved from genome-wide association studies (GWAS) [35].

Genomic databases such as the 1000 Genomes Project (http://browser.1000genomes.org), the Trans-Omics for Precision Medicine Program (TOPMed; https://www.nhlbiwgs.org/), or The Genome Aggregation Database (gnomAD; https://gnomad.broadinstitute.org/) compile data with a few to several thousand of participants from different ethnic groups from large sequencing consortiums. Nonetheless, limitations like low-quality data, lack of details on the origin of studies, or absence of information regarding possible associated phenotypes can impact variant interpretation [36]. In line with this, low (<1%) or very low (<0.1%) population frequencies cannot exclude pathogenicity per se, in particular when clinical and experimental evidence support the deleterious nature of the variant. Cancer syndromes, including HDGC and hereditary lobular breast cancer, occur during adulthood and do not affect reproductive success, thus allowing transmission of deleterious variants across populations. More so, missense mutations are subtle alterations in genetic terms, often resulting in mild effects on protein level and activity, which is reflected in low penetrance and less striking phenotypes [31, 37]. Despite the controversy surrounding their functional significance over the years, it is undeniable that pathogenic missense variants result in clinical manifestations indistinguishable from those caused by truncating mutations [9, 38].

6.4.2 In Silico Models

Computational applications have been projected into the spotlight due to their usefulness in predicting the degree of conservation of mutated amino acids within species, their impact on splicing and, ultimately, on protein structure [6, 31]. Pathogenicity predictions are usually based on homology concepts, assuming that amino acids conserved across different species are relevant and that their substitution is likely to compromise protein function [31, 39]. Focusing on *CDH1* missense alterations, standard tools encompass SIFT (Sorting Intolerant from Tolerant; http://sift.jcvi.org/) [40]; PolyPhen-2 (Polymorphism Phenotyping v2; http://genetics.bwh.harvard.edu/pph2/) [41]; and PROVEAN (Protein Variation Effect

Analyzer; http://provean.jcvi.org) [42]. The use of multiple programs is indeed highly recommended as different outputs can be obtained, depending on the algorithm under testing [36]. One major drawback of in silico models is that the significance of each amino acid is considered independently and, as such, they do not contemplate compensatory effects of neighbouring positions [31, 43]. To overcome this limitation, structural models of E-cadherin have been developed and currently cover the prodomain, the extracellular, and the catenin-binding domains [39]. Structural-based estimations can be obtained through calculation of protein native-state stability changes using FoldX (http://foldxsuite.crg.eu/) [44]. Specifically, an energetic difference between the mutant and the wild-type reference greater than 0.8 kcal/mol indicates a destabilized protein structure. Variants that induce higher energetic penalties have been correlated with in vitro loss of function and with a younger age at diagnosis or death by diffuse gastric cancer [33, 39]. Unfortunately, models were built using *Xenopus* and mouse data and, therefore, prediction performance is restricted to regions with reliable alignment with the human sequence. Furthermore, due to low structural content of the juxtamembrane domain, no model is available for accurate energy calculations and, as a consequence, mutations affecting this region cannot be analysed.

Additional analyses may include the study of alternative splicing and processing of introns in nuclear pre-mRNA using the Netgene2 algorithm (http://www.cbs.dtu.dk/services/NetGene2/) [45, 46]. Nevertheless, the identification of cryptic splice sites in the *CDH1* gene is limited to available transcriptional data and requires molecular validation [34].

6.4.3 In Vitro Assays

In the last two decades, Seruca's group has been devoted to improve the assessment of the functional significance of *CDH1* variants [10, 47–50]. In this context, several experimental assays have been developed to characterize variant effects at the cellular level, namely E-cadherin expression and localization, cell-cell adhesive function, invasive profile, and cell topology. The pipeline is streamlined and takes advantage of an immortalized E-cadherin negative cell line (Chinese Hamster Ovary—CHO) transfected with vectors encoding wild-type and mutant E-cadherin forms subsequently analysed through Western Blot, immunofluorescence, bioimaging applications, slow aggregation, and matrigel invasion assays [33, 51].

According to this protocol, variants inducing low E-cadherin levels may reveal severe protein defects and activation of protein quality control mechanisms, culminating in premature degradation of E-cadherin and absence of functional activity [33, 39, 52]. A band mobility shift is seen on occasions, suggesting that the variant may affect protein glycosylation—a pivotal process for E-cadherin folding, trafficking, and stability at the plasma membrane [34, 53].

By coupling immunostaining with advanced bioimaging techniques, it has been possible to provide a faithful characterization of E-cadherin expression patterns, thus enabling the discrimination of deleterious and neutral variants [49, 54]. In contrast to

wild-type E-cadherin, which is mainly expressed at the plasma membrane, deleterious variants can be diffusely distributed throughout the cell or present aberrant signal accumulation in cytoplasmic regions/organelles [49]. Interestingly, some deleterious variants are expressed at the plasma membrane but affect the stability of homophilic cadherin linkages on neighbouring cells, resulting in increased protein turnover rates [48, 49, 55]. Fluorescence microscopy images are further explored to compare intercellular organization and morphological aspects of cells expressing wild-type and mutant E-cadherin [50]. In this regard, cells expressing wild-type and dysfunctional variants yield very different cell networks, with the latter composed by triangles with bigger areas and edges indicative of cell-cell loosening and cytoplasm extension [50].

To evaluate the ability of establishing cell-cell adhesion, slow aggregation assays are mostly used due to their simplicity and consistency among replicates. Herein, cells with a competent adhesion complex spontaneously aggregate upon seeding on a semi-solid agar substrate [56]. In contrast, cells expressing dysfunctional E-cadherin present an isolated phenotype or form small cellular aggregates with different cohesion degrees as verified by quantification of aggregate area and density [33, 47, 48, 57].

Ultimately, the invasion-suppressor role of E-cadherin is investigated through matrigel invasion chambers. Wild-type and mutant cells are seeded on top of a device coated with matrigel, an artificial ECM whose composition resembles that of the basement membrane [58, 59]. Cells able to degrade the matrix layer and reach the lower side of the device are classified as invasive, while those remaining on top of the matrigel are considered non-invasive [10].

6.4.4 In Vivo Approaches

Having settled cellular and functional processes using in vitro systems, in vivo strategies become essential for concept validation and guidance towards translational research. Although frequently used in in vivo studies, mice models present several drawbacks in the context of gastric cancer [60–62], prompting research in alternative living systems.

Drosophila melanogaster has emerged as a valuable model organism to study the molecular mechanisms and genetic processes triggered by E-cadherin missense variants, mainly owing to its versatility and genetically tractable options [63].

The generation of fly lines carrying HDGC-associated variants was first reported in 2006 [64]. At that time, a GAL4/UAS system was used to induce expression of two E-cadherin missense variants in the *Drosophila*-developing wing epithelium, the so-called wing imaginal disc. By inspection of this simple monolayer epithelium, it was verified that cells expressing A634V and V832M variants infiltrate neighbouring regions, in contrast to cells expressing wild-type E-cadherin that maintain their epithelial morphology and adhesive properties [64]. Of note, the two E-cadherin mutants showed distinct patterns of invasion, with the A634V

extracellular mutant invading collectively whereas the V832M intracellular mutant invaded in an isolated manner [64].

In addition to this system, the *Drosophila* ovary has also been exploited to evaluate variant impact on epithelial organization and on border cell migration during oogenesis [33, 65]. Figueiredo et al. have engineered a model in which the G212E variant was expressed in the *Drosophila* follicular epithelium, enabling direct comparison between expressing and non-expressing clones within a mosaic tissue [33]. The authors observed that clones expressing the G212E mutant induce epithelial invaginations and disrupt tissue architecture and integrity [33]. Further, the apical marker aPKC was found to be decreased in G212E-expressing cells, when compared with non-expressing tissue, confirming loss of apical-basal polarity [33].

An innovative model was designed to demonstrate the damaging nature of the R749W variant. By monitoring migration dynamics of border cells overexpressing wild-type and mutant E-cadherin forms across the *Drosophila* germline, it could be seen that wild-type E-cadherin cells travel only 49% of the expected distance, whereas the motile performance of cells expressing the R749W mutant is similar to that of cells expressing an inert UAS-driven transgene [65].

In conclusion, in vivo models have been a breakthrough for the management of *CDH1* variant carriers and their families, as well as for research applications addressing molecular and cellular cues taking into consideration the tissue environment [33, 66].

6.5 Molecular Mechanisms Triggered by *CDH1* Variants

Aside from a great potential in pathological assessment, it is indisputable that experimental approaches have been contributing to the understanding of basic mechanisms and molecular features underlying E-cadherin dysfunction in disease.

Evidence has revealed that the effects of E-cadherin variants may take place upon translation initiation. Variants affecting just the signal peptide of E-cadherin, without changing the remaining predicted sequence, were found to impair the binding of cellular components that are crucial for protein translation and subsequent translocation into the endoplasmic reticulum (ER, Fig. 6.2) [51].

The ER is the site of maturation for secretory and membrane proteins and the synthetic output of this organelle is tightly controlled, certifying both quality and quantity of emergent proteins. In 2008, it was first described that E-cadherin protein levels and activity are critically modulated by mechanisms of protein quality control associated with ER (endoplasmic reticulum-associated degradation, ERAD), and therefore their subversion could be an alternative mechanism for E-cadherin loss during cancer progression [52]. Accordingly, R749W and E757K variants affecting the juxtamembrane region of E-cadherin generated misfolded proteins, which were recognized and promptly degraded by the proteasome [52]. In a subsequent study, DNAJB4 molecular chaperone was pinpointed as an important mediator of this checkpoint by distinguishing wild-type from mutant proteins, thus determining their fate both in vitro and in vivo [67]. Remarkably, it was demonstrated that

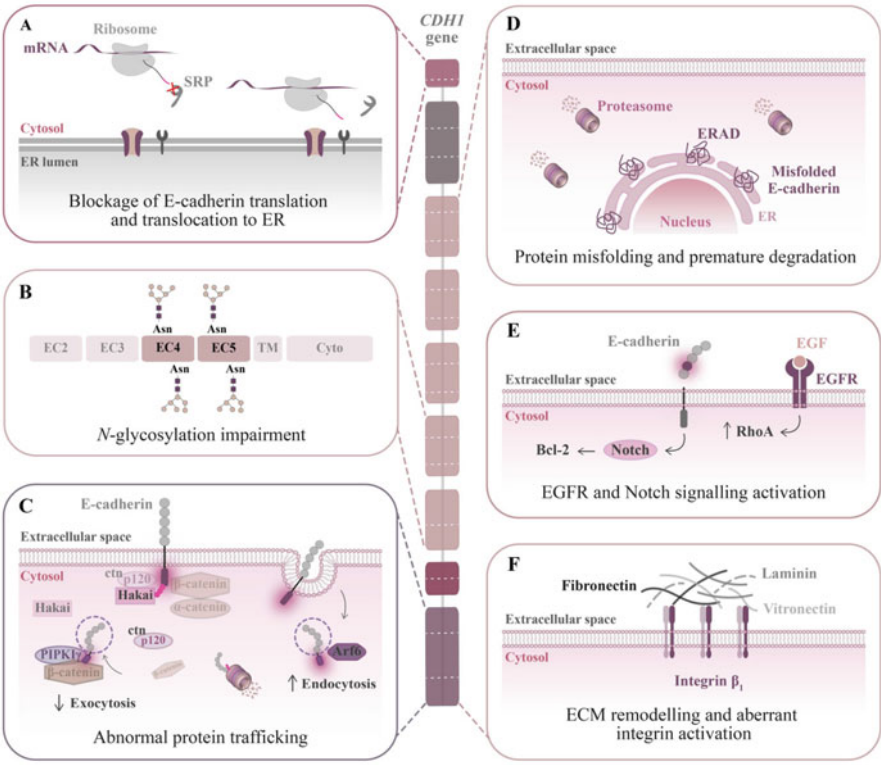


Fig. 6.2 Molecular mechanisms underlying pathogenicity of *CDH1* variants. (**a**) Variants affecting the signal peptide of E-cadherin may impair protein translation and translocation into the endoplasmic reticulum (ER). (**b**) Irregular patterns of E-cadherin at the plasma membrane and diffuse cellular aggregates may arise due to abnormal protein glycosylation. (**c**) Disruption of the binding site of various partners, namely p120, β-catenin or type I gamma phosphatidylinositol phosphate kinase (PIPKIγ), compromises E-cadherin trafficking and function. (**d**) E-cadherin mutations yield misfolded proteins, which are recognized by mechanisms of protein quality control, leading to their premature degradation. (**e**) Loss of E-cadherin activates EGFR and Notch signalling pathways awarding cells increased motile and survival abilities. (**f**) Aberrant integrin activation is associated with increased cell-ECM interaction and dissemination of E-cadherin mutant cells. Schematic representation of E-cadherin highlighting protein domains associated with altered molecular mechanisms

chemical chaperones such as dimethyl sulfoxide (DMSO) and 4-phenylbutyrate (4-PBA) may assist the folding of missense mutant forms and even rescue E-cadherin functional activity, emerging as promising therapeutic approaches in patients harbouring missense variants [52, 68].

Occasionally, missense variants disrupt *NX(S/T)* consensus sequences, where *X* can be any amino acid with the exception of proline, interfering with the transfer of *N*-glycans to the side chain of asparagine (*N*) [53, 69]. For instance, it was verified that the T560R variant impairs *N*-glycosylation in E-cadherin's fourth extracellular

domain (EC4), resulting in an irregular pattern of E-cadherin at the plasma membrane and in diffuse cellular aggregates on an agar substrate, illustrative of immature protein forms [34].

Variants affecting the intracellular portion of E-cadherin are also a dilemma for trafficking pathways, since this region serves as a binding site for a set of partners responsible for protein exocytosis and endocytosis [48]. Specifically, a weak interaction between E-cadherin and either β-catenin or type I gamma phosphatidylinositol phosphate kinase (PIPKIγ) decreases the quantity of E-cadherin molecules trafficked from the Golgi to the plasma membrane. In contrast, in the absence of p120-catenin linkages, E-cadherin becomes available to be targeted by Hakai, leading to its ubiquitination and degradation [48].

Attesting the importance of E-cadherin as a signalling molecule, a number of variants have been associated with increased activation of EGFR and its downstream effectors, including RhoA, Src kinase and p38 MAPK [70, 71]. Of note, these signals correlate with motile capabilities and formation of filopodia, lamellipodia and other cytoskeletal structures [55, 70, 71]. Along with dissemination skills, E-cadherin dysfunction awards cells increased resistance to apoptosis through a Notch-dependent upregulation of Bcl-2 [72, 73].

More recently, research has been focusing on the dynamic interplay between E-cadherin mutant cells and the ECM. It is documented that loss of E-cadherin induces increased secretion and deposition of Laminin γ2, promoting evasion of cell death and invasion of tissues [66]. Furthermore, cells expressing E-cadherin missense variants exhibited enhanced ECM adhesiveness and higher traction forces in substrates combining fibronectin and collagen IV, collagen VI or laminin. This bias on ECM preference suggested the activation of Integrin β1, which integrates the most promiscuous class of integrins [65]. Consistent with this, depletion of Integrin β1 increased cell-cell cohesion and hindered invasion of all E-cadherin mutants tested. The E-cadherin/Integrin β1 crosstalk was validated in a *Drosophila* model and in transcriptomic data of 262 gastric carcinoma cases retrieved from the cancer genome atlas (TCGA) [65]. Loss of E-cadherin and increased Integrin β1 expression were found associated with advanced tumour grade and poor patient overall survival in primary gastric cancer [65].

All these findings have certainly shed light on the severe effects caused by loss of E-cadherin expression due to pathogenic *CDH1* mutations, and sparked research on the development of innovative therapeutic strategies and prognostic biomarkers. Ultimately, we expect to be able to rescue the aggressive behaviour elicited by E-cadherin dysfunction, improving patient management and overall survival.

6.6 Conclusion and Future Challenges

It is clear that pathogenic *CDH1* missense alterations result in tissue damage, a hallmark of cancer and congenital abnormalities. These deleterious effects may arise following deregulation of intracellular signalling, activation of oncogenic pathways and abnormal interaction with the ECM. Thus, we propose that there should be a concerted approach to evaluate the significance of missense alterations, which

should comprise functional data, disease-specific criteria and mutation geographic distribution. In this sense, a critical issue will be the development of improved guidelines to estimate the accurate risk of cancer and/or developmental malformations for patients bearing *CDH1* missense variants.

In the future, we should be dedicated to groundbreaking research programs to unveil the paradigm surrounding *CDH1*/E-cadherin pleiotropy. We postulate that, by addressing the ECM composition of those tissues most frequently associated with *CDH1*-mediated disorders, we may expose molecular, microenvironmental and genetic factors determinant for the manifestation of distinct clinical phenotypes.

Acknowledgements We would like to pay a tribute to Raquel Seruca, our forever inspiring mentor. To infinity and beyond.

Funding This work was financed by FEDER funds through the Operational Programme for Competitiveness Factors (COMPETE 2020), Programa Operacional de Competitividade e Internacionalização (POCI) and Programa Operacional Regional do Norte (Norte 2020); and by National Funds through the Portuguese Foundation for Science and Technology (FCT projects PTDC/MED-GEN/30356/2017, EXPL/MED-ONC/0386/2021, 2022.02665.PTDC, and doctoral grant SFRH/BD/143533/2019-JP). We acknowledge the American Association of Patients with Hereditary Gastric Cancer "No Stomach for Cancer" for funding Seruca's research, and the project "P.CCC: Centro Compreensivo de Cancro do Porto" - NORTE-01-0145-FEDER-072678, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

References

1. Oliveira C et al (2015) Familial gastric cancer: genetic susceptibility, pathology, and implications for management. Lancet Oncol 16(2):e60–e70
2. Guilford P et al (1998) E-cadherin germline mutations in familial gastric cancer. Nature 392(6674):402–405
3. Hansford S et al (2015) Hereditary diffuse gastric cancer syndrome: CDH1 mutations and beyond. JAMA Oncol 1(1):23–32
4. Blair VR et al (2020) Hereditary diffuse gastric cancer: updated clinical practice guidelines. Lancet Oncol 21(8):e386–e397
5. Gamble LA, Heller T, Davis JL (2021) Hereditary diffuse gastric cancer syndrome and the role of CDH1 a review. JAMA Surg 156(4):387–392
6. van der Post RS et al (2015) Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. J Med Genet 52(6):361–374
7. Gamble LA, Davis JL (2022) Surveillance and surgical considerations in hereditary diffuse gastric cancer. Gastrointest Endosc Clin N Am 32(1):163–175
8. Caldas C et al (1999) Familial gastric cancer: overview and guidelines for management. J Med Genet 36(12):873–880
9. Figueiredo J et al (2019) Clinical spectrum and pleiotropic nature of CDH1 germline mutations. J Med Genet 56(4):199–208
10. Melo S et al (2017) Predicting the functional impact of CDH1 missense mutations in hereditary diffuse gastric cancer. Int J Mol Sci 18(12)
11. Corso G et al (2012) Frequency of CDH1 germline mutations in gastric carcinoma coming from high- and low-risk areas: metanalysis and systematic review of the literature. BMC Cancer 12:8

12. Lecuit T, Yap AS (2015) E-cadherin junctions as active mechanical integrators in tissue dynamics. Nat Cell Biol 17(5):533–539
13. van Roy F, Berx G (2008) The cell-cell adhesion molecule E-cadherin. Cell Mol Life Sci 65(23):3756–3788
14. DuFort CC, Paszek MJ, Weaver VM (2011) Balancing forces: architectural control of mechanotransduction. Nat Rev Mol Cell Biol 12(5):308–319
15. Rubsam M et al (2017) E-cadherin integrates mechanotransduction and EGFR signaling to control junctional tissue polarization and tight junction positioning. Nat Commun 8(1):1250
16. Corso G et al (2018) Hereditary lobular breast cancer with an emphasis on E-cadherin genetic defect. J Med Genet 55(7):431–441
17. Oliveira C et al (2013) E-cadherin alterations in hereditary disorders with emphasis on hereditary diffuse gastric cancer. Prog Mol Biol Transl Sci 116:337–359
18. Molinaro V et al (2014) Complementary molecular approaches reveal heterogeneous CDH1 germline defects in Italian patients with hereditary diffuse gastric cancer (HDGC) syndrome. Genes Chromosomes Cancer 53(5):432–445
19. Schrader KA et al (2011) Germline mutations in CDH1 are infrequent in women with early-onset or familial lobular breast cancers. J Med Genet 48(1):64–68
20. Vogelaar IP et al (2013) Identification of germline mutations in the cancer predisposing gene CDH1 in patients with orofacial clefts. Hum Mol Genet 22(5):919–926
21. Ghoumid J et al (2017) Blepharocheilodontic syndrome is a CDH1 pathway-related disorder due to mutations in CDH1 and CTNND1. Genet Med 19(9):1013–1021
22. Kievit A et al (2018) Variants in members of the cadherin-catenin complex, CDH1 and CTNND1, cause blepharocheilodontic syndrome. Eur J Hum Genet 26(2):210–219
23. Corso G et al (2021) Geographical distribution of E-cadherin germline mutations in the context of diffuse gastric cancer: a systematic review. Cancers (Basel) 13(6)
24. Sung H et al (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71(3):209–249
25. Guilford PJ et al (1999) E-cadherin germline mutations define an inherited cancer syndrome dominated by diffuse gastric cancer. Hum Mutat 14(3):249–255
26. Corso G et al (2022) Global distribution of prophylactic total gastrectomy in E-cadherin (CDH1) mutations. Semin Oncol 49:130
27. Corso G et al (2020) Hereditary gastric and breast cancer syndromes related to CDH1 germline mutation: a multidisciplinary clinical review. Cancers (Basel) 12(6)
28. Santucci C et al (2020) Progress in cancer mortality, incidence, and survival: a global overview. Eur J Cancer Prev 29(5):367–381
29. Lee K et al (2018) Specifications of the ACMG/AMP variant curation guidelines for the analysis of germline CDH1 sequence variants. Hum Mutat 39(11):1553–1568
30. Figueiredo J, Seruca J (2013) Germline missense mutants in hereditary diffuse gastric cancer. Spotlight Familial Hereditary Gastric Cancer 7:77–86
31. Suriano G et al (2006) A model to infer the pathogenic significance of CDH1 germline missense variants. J Mol Med 84(12):1023–1031
32. Fitzgerald RC, Caldas C (2004) Clinical implications of E-cadherin associated hereditary diffuse gastric cancer. Gut 53(6):775–778
33. Figueiredo J et al (2021) Germline CDH1 G212E missense variant: combining clinical. In vitro and in vivo strategies to unravel disease burden. Cancers (Basel) 13(17)
34. Pena-Couso L et al (2018) Clinical and functional characterization of the CDH1 germline variant c.1679C>G in three unrelated families with hereditary diffuse gastric cancer. Eur J Hum Genet 26(9):1348–1353
35. Gibson G (2012) Rare and common variants: twenty arguments. Nat Rev Genet 13(2): 135–145
36. Richards S et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17(5):405–424

37. Zhang L et al (2014) The germline CDH1 c.48 G>C substitution contributes to cancer predisposition through generation of a pro-invasive mutation. Mutat Res 770:106–111
38. Barber ME et al (2008) Histopathological and molecular analysis of gastrectomy specimens from hereditary diffuse gastric cancer patients has implications for endoscopic surveillance of individuals at risk. J Pathol 216(3):286–294
39. Simoes-Correia J et al (2012) E-cadherin destabilization accounts for the pathogenicity of missense mutations in hereditary diffuse gastric cancer. PLoS One 7(3):e33783
40. Kumar P, Henikoff S, Ng PC (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 4(7):1073–1081
41. Adzhubei IA et al (2010) A method and server for predicting damaging missense mutations. Nat Methods 7(4):248–249
42. Choi Y et al (2012) Predicting the functional effect of amino acid substitutions and indels. PLoS One 7(10):e46688–e46688
43. Saunders CT, Baker D (2002) Evaluation of structural and evolutionary contributions to deleterious mutation prediction. J Mol Biol 322(4):891–901
44. Schymkowitz J et al (2005) The FoldX web server: an online force field. Nucleic Acids Res 33 (Web Server issue):W382–W388
45. Brunak S, Engelbrecht J, Knudsen S (1991) Prediction of human mRNA donor and acceptor sites from the DNA sequence. J Mol Biol 220(1):49–65
46. Hebsgaard SM et al (1996) Splice site prediction in Arabidopsis thaliana pre-mRNA by combining local and global sequence information. Nucleic Acids Res 24(17):3439–3452
47. Suriano G et al (2003) Identification of CDH1 germline missense mutations associated with functional inactivation of the E-cadherin protein in young gastric cancer probands. Hum Mol Genet 12(5):575–582
48. Figueiredo J et al (2013) The importance of E-cadherin binding partners to evaluate the pathogenicity of E-cadherin missense mutations associated to HDGC. Eur J Hum Genet 21(3):301–309
49. Sanches JM et al (2015) Quantification of mutant E-cadherin using bioimaging analysis of in situ fluorescence microscopy. A new approach to CDH1 missense variants. Eur J Hum Genet 23(8):1072–1079
50. Mestre T et al (2016) Quantification of topological features in cell meshes to explore E-cadherin dysfunction. Sci Rep 6:25101
51. Figueiredo J et al (2018) E-cadherin signal sequence disruption: a novel mechanism underlying hereditary cancer. Mol Cancer 17(1):112
52. Simoes-Correia J et al (2008) Endoplasmic reticulum quality control: a new mechanism of E-cadherin regulation and its implication in cancer. Hum Mol Genet 17(22):3566–3576
53. Carvalho S et al (2016) Preventing E-cadherin aberrant N-glycosylation at Asn-554 improves its critical function in gastric cancer. Oncogene 35(13):1619–1631
54. Figueiredo J et al (2018) Geometric compensation applied to image analysis of cell populations with morphological variability: a new role for a classical concept. Sci Rep 8(1): 10266
55. Suriano G et al (2003) E-cadherin germline missense mutations and cell phenotype: evidence for the independence of cell invasion on the motile capabilities of the cells. Hum Mol Genet 12(22):3007–3016
56. Boterberg T et al (2001) Cell aggregation assays. Methods Mol Med 58:33–45
57. Kluijt I et al (2011) CDH1-related hereditary diffuse gastric cancer syndrome: clinical variations and implications for counseling. Int J Cancer
58. Kleinman HK, Martin GR (2005) Matrigel: basement membrane matrix with biological activity. Semin Cancer Biol 15(5):378–386
59. Kleinman HK et al (1982) Isolation and characterization of type IV procollagen, laminin, and heparan sulfate proteoglycan from the EHS sarcoma. Biochemistry 21(24):6188–6193
60. Humar B et al (2009) E-cadherin deficiency initiates gastric signet-ring cell carcinoma in mice and man. Cancer Res 69(5):2050–2056

61. Mimata A et al (2011) Loss of E-cadherin in mouse gastric epithelial cells induces signet ring-like cells, a possible precursor lesion of diffuse gastric cancer. Cancer Sci 102(5):942–950
62. Shimada S et al (2012) Synergistic tumour suppressor activity of E-cadherin and p53 in a conditional mouse model for metastatic diffuse-type gastric cancer. Gut 61(3):344–353
63. Caldeira J et al (2009) Using fruitflies to help understand the molecular mechanisms of human hereditary diffuse gastric cancer. Int J Dev Biol 53(8–10):1557–1561
64. Pereira PS et al (2006) E-cadherin missense mutations, associated with hereditary diffuse gastric cancer (HDGC) syndrome, display distinct invasive behaviors and genetic interactions with the Wnt and notch pathways in Drosophila epithelia. Hum Mol Genet 15(10):1704–1712
65. Figueiredo J et al (2022) Integrin beta1 orchestrates the abnormal cell-matrix attachment and invasive behaviour of E-cadherin dysfunctional cells. Gastric Cancer 25(1):124–137
66. Caldeira J et al (2015) E-cadherin-defective gastric cancer cells depend on laminin to survive and invade. Hum Mol Genet 24(20):5891–5900
67. Simoes-Correia J et al (2014) DNAJB4 molecular chaperone distinguishes WT from mutant E-cadherin, determining their fate in vitro and in vivo. Hum Mol Genet 23(8):2094–2105
68. Figueiredo J et al (2011) ADP-ribosylation factor 6 mediates E-cadherin recovery by chemical chaperones. PLoS One 6(8):e23188
69. Pinho SS et al (2011) Modulation of E-cadherin function and dysfunction by N-glycosylation. Cell Mol Life Sci 68(6):1011–1020
70. Mateus AR et al (2007) EGFR regulates RhoA-GTP dependent cell motility in E-cadherin mutant cells. Hum Mol Genet 16(13):1639–1647
71. Mateus AR et al (2009) E-cadherin mutations and cell motility: a genotype-phenotype correlation. Exp Cell Res 315(8):1393–1402
72. Ferreira P et al (2005) Loss of functional E-cadherin renders cells more resistant to the apoptotic agent taxol in vitro. Exp Cell Res 310(1):99–104
73. Ferreira AC et al (2012) E-cadherin impairment increases cell survival through notch-dependent upregulation of Bcl-2. Hum Mol Genet 21(2):334–343
74. Guilford P, Humar B, Blair V (2010) Hereditary diffuse gastric cancer: translation of CDH1 germline mutations into clinical practice. Gastric Cancer 13(1):1–10
75. Suriano G et al (2005) Characterization of a recurrent germ line mutation of the E-cadherin gene: implications for genetic testing and clinical management. Clin Cancer Res 11(15):5401–5409
76. Shinmura K et al (1999) Familial gastric cancer: clinicopathological characteristics, RER phenotype and germline p53 and E-cadherin mutations. Carcinogenesis 20(6):1127–1131
77. Guindalini RSC et al (2019) Frequency of CDH1 germline variants and contribution of dietary habits in early age onset gastric cancer patients in Brazil. Gastric Cancer 22(5):920–931
78. More H et al (2007) Identification of seven novel germline mutations in the human E-cadherin (CDH1) gene. Hum Mutat 28(2):203
79. Avizienyte E et al (2001) E-cadherin is not frequently mutated in hereditary gastric cancer. J Med Genet 38(1):49–52
80. Corso G et al (2011) E-cadherin genetic screening and clinico-pathologic characteristics of early onset gastric cancer. Eur J Cancer 47(4):631–639
81. Petrova YI, Schecterson L, Gumbiner BM (2016) Roles for E-cadherin cell surface regulation in cancer. Mol Biol Cell 27(21):3233–3244
82. Kaurah P et al (2007) Founder and recurrent CDH1 mutations in families with hereditary diffuse gastric cancer. JAMA 297(21):2360–2372
83. Kim S et al (2013) Searching for E-cadherin gene mutations in early onset diffuse gastric cancer and hereditary diffuse gastric cancer in Korean patients. Familial Cancer 12(3): 503–507
84. Yoon KA et al (1999) Germline mutations of E-cadherin gene in Korean familial gastric cancer patients. J Hum Genet 44(3):177–180
85. Ikonen T et al (2001) Association of E-cadherin germ-line alterations with prostate cancer. Clin Cancer Res 7(11):3465–3471

86. Brooks-Wilson AR et al (2004) Germline E-cadherin mutations in hereditary diffuse gastric cancer: assessment of 42 new families and review of genetic screening criteria. J Med Genet 41(7):508–517
87. Betes M et al (2017) A multidisciplinary approach allows identification of a new pathogenic CDH1 germline missense mutation in a hereditary diffuse gastric cancer family. Dig Liver Dis 49(7):825–826
88. Oliveira C et al (2002) Screening E-cadherin in gastric cancer families reveals germline mutations only in hereditary diffuse gastric cancer kindred. Hum Mutat 19(5):510–517
89. Corso G et al (2007) Characterization of the P373L E-cadherin germline missense mutation and implication for clinical management. Eur J Surg Oncol 33(9):1061–1067
90. Wang Y et al (2003) Ile-Leu substitution (I415L) in germline E-cadherin gene (CDH1) in Japanese familial gastric cancer. Jpn J Clin Oncol 33(1):17–20
91. Keller G et al (2004) Germline mutations of the E-cadherin(CDH1) and TP53 genes, rather than of RUNX3 and HPP1, contribute to genetic predisposition in German gastric cancer patients. J Med Genet 41(6):e89
92. Ascano JJ et al (2001) Inactivation of the E-cadherin gene in sporadic diffuse-type gastric cancer. Mod Pathol 14(10):942–949
93. Adib E et al (2022) CDH1 germline variants are enriched in patients with colorectal cancer, gastric cancer, and breast cancer. Br J Cancer 126(5):797–803
94. Chen QH et al (2013) Novel CDH1 germline mutations identified in Chinese gastric cancer patients. World J Gastroenterol 19(6):909–916
95. Garziera M et al (2013) A novel CDH1 germline missense mutation in a sporadic gastric cancer patient in north-east of Italy. Clin Exp Med 13(2):149–157
96. Garziera M et al (2013) Identification and characterization of CDH1 germline variants in sporadic gastric cancer patients and in individuals at risk of gastric cancer. PLoS One 8(10): e77035
97. Sarrio D et al (2003) Epigenetic and genetic alterations of APC and CDH1 genes in lobular breast cancer: relationships with abnormal E-cadherin and catenin expression and microsatellite instability. Int J Cancer 106(2):208–215
98. Stuebs F et al (2018) CDH1 mutation screen in a BRCA1/2-negative familial breast-/ovarian cancer cohort. Arch Gynecol Obstet 297(1):147–152
99. Kim HC et al (2000) The E-cadherin gene (CDH1) variants T340A and L599V in gastric and colorectal cancer patients in Korea. Gut 47(2):262–267
100. Salahshor S et al (2001) A germline E-cadherin mutation in a family with gastric and colon cancer. Int J Mol Med 8(4):439–443
101. Jonsson BA et al (2002) Germline mutations in E-cadherin do not explain association of hereditary prostate cancer, gastric cancer and breast cancer. Int J Cancer 98(6):838–843
102. Forster A et al (2021) Rare germline variants in the E-cadherin gene CDH1 are associated with the risk of brain tumors of neuroepithelial and epithelial origin. Acta Neuropathol 142(1): 191–210
103. Du S et al (2019) A novel CDH1 mutation causing reduced E-cadherin dimerization is associated with nonsyndromic cleft lip with or without cleft palate. Genet Test Mol Biomarkers 23(11):759–765
104. Cox LL et al (2018) Mutations in the epithelial cadherin-p120-catenin complex cause Mendelian non-syndromic cleft lip with or without cleft palate. Am J Hum Genet 102(6):1143–1157
105. Brito LA et al (2015) Rare variants in the epithelial cadherin gene underlying the genetic etiology of nonsyndromic cleft lip with or without cleft palate. Hum Mutat 36(11):1029–1033
106. Ittiwut R et al (2016) Variants of the CDH1 (E-cadherin) gene associated with oral clefts in the Thai population. Genet Test Mol Biomarkers 20(7):406–409
107. Nishi E et al (2016) Exome sequencing-based identification of mutations in non-syndromic genes among individuals with apparently syndromic features. Am J Med Genet A 170(11): 2889–2894

Other Syndromes and Genes Associated with Gastric Cancer Predisposition

7

Gianluca Tedaldi, Celina São José, and Carla Oliveira

Abstract

Gastric cancer is the fifth most common type of cancer and the fourth leading cause of cancer-related death; nevertheless, genetic predisposition to this malignancy is still widely unexplored.

Besides hereditary diffuse gastric cancer (HDGC), associated with germline *CDH1* and *CTNNA1* pathogenic variants, other genetic syndromes characterized by high risk to develop gastric cancer have been described, encompassing gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS), associated with germline genetic variants in the *APC* promoter, and familial intestinal gastric cancer (FIGC), still lacking a clear genetic cause.

Moreover, gastric cancer risk is associated with pathogenic variants in genes involved in DNA mismatch repair, such as *MLH1* and *MSH2* (Lynch syndrome), apoptosis, including *TP53* (Li-Fraumeni syndrome) and double-strand break

G. Tedaldi (✉)
Biosciences Laboratory, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy
e-mail: gianluca.tedaldi@irst.emr.it

C. São José
i3S, Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal

IPATIMUP, Institute of Molecular Pathology and Immunology, University of Porto, Porto, Portugal

Doctoral Programme in Biomedicine, Faculty of Medicine, University of Porto, Porto, Portugal

C. Oliveira
i3S, Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal

IPATIMUP, Institute of Molecular Pathology and Immunology, University of Porto, Porto, Portugal

FMUP - Faculty of Medicine of the University of Porto, Porto, Portugal

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_7

repair, such as *BRCA1*/*BRCA2* and *PALB2* (hereditary breast and ovarian cancer syndrome).

Furthermore, gastric cancer can be a manifestation of gastrointestinal polyposis syndromes, such as those associated with *APC* (familial adenomatous polyposis), *MUTYH* (MUTYH-associated polyposis), *BMPR1A*/*SMAD4* (juvenile polyposis syndrome), *STK11* (Peutz-Jeghers syndrome), and *PTEN* (Cowden syndrome) genes.

Recent advances in molecular techniques, such as next-generation sequencing, led to the identification of many new genes involved in the predisposition to gastric cancer, some of which are low or moderate penetrant that predispose to other syndromes.

Consequently, in patients with early onset gastric cancer and/or strong gastric cancer family history, the use of multigene panel testing should be considered in cancer risk assessment, including different surveillance recommendations for each syndrome.

7.1 Introduction

Familial predisposition to gastric cancer (GC) has been categorized into three main syndromes with primary predisposition to the stomach: (1) hereditary diffuse gastric cancer (HDGC), (2) familial intestinal gastric cancer (FIGC), and (3) gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS). While tumor burden and main genetic causality are established for HDGC (*CDH1*, *CTNNA1*) and GAPPS (*APC*), FIGC remains genetically unexplained and understudied. Nevertheless, other genes that predispose for other cancer syndromes encompass GC within their tumor spectrum: (1) *MLH1*, *MSH2*, *MSH6*, and *PMS2* (Lynch syndrome, LS, or hereditary nonpolyposis colorectal cancer, HNPCC), (2) *TP53* (Li-Fraumeni syndrome, LFS), (3) *BRCA1*, *BRCA2*, and *PALB2* (hereditary breast and ovarian cancer, HBOC), (4) *APC* (familial adenomatous polyposis, FAP), (5) *MUTYH* (MUTYH-associated polyposis, MAP), (6) *BMPR1A* and *SMAD4* (juvenile polyposis syndrome, JPS), (7) *STK11* (Peutz-Jeghers syndrome, PJS), and (8) *PTEN* (PTEN Hamartoma Tumor syndrome, PHTS) (Table 7.1) [1].

7.2 HDGC

Pathogenic or likely pathogenic variants in *CDH1* predispose to HDGC, an autosomal dominant syndrome characterized by diffuse gastric cancer (DGC) and lobular breast cancer (LBC) [2].

In recent years, next-generation sequencing (NGS) approaches have evolved exponentially, leading to the identification of new genes in HDGC. In 2013, the first germline truncating variant in *CTNNA1*, encoding the α-E-catenin protein, was described in an HDGC family [3]. To date, and after multiple HDGC families being

Table 7.1 Hereditary syndromes associated with GC

Syndrome	Gene	GC risk (%)	References
GAPPS	*APC (promoter 1B)*	IGC 13%	[18]
FIGC	Probably polygenic	Variable	[19]
LS	*MLH1*	IGC 5–10%	[20–24]
	MSH2	IGC 9%	[23, 24]
	MSH6	IGC ≤1%–7.9%	[23–26]
	PMS2	Low	[23, 24]
	EPCAM	Low	[24, 27]
LFS	*TP53*	IGC or DGC 2–5%	[28, 29]
HBOC	*BRCA1/BRCA2*	IGC 2%	[29, 30]
FAP	*APC*	IGC 4–7% (Asian population), low (Western population)	[24, 31]
MAP	*MUTYH*	IGC 2–5%	[24, 32]
PJS	*STK11*	IGC 29%	[24, 33]
JPS	*SMAD4/BMPR1A*	IGC or DGC 10–30%	[24, 34]
CS	*PTEN*	Low	[29, 35]

IGC: intestinal-type gastric cancer
DGC: diffuse-type gastric cancer

identified to carry *CTNNA1* truncating variants, *CTNNA1* remains the only gene, besides *CDH1*, clearly associated with the HDGC syndrome [4, 5]. Germline mutations in *MAP3K6* and *MYD88* have also been reported in HDGC families [6, 7]; however, the specific role of these genes remains unclear and their involvement in GC predisposition is still questionable [4]. In 2015, a targeted analysis with a panel of 55 cancer-related genes performed on 144 *CDH1*-negative cases found candidate mutations in 16 probands (11%), including high and moderate penetrance mutations in *CTNNA1*, *BRCA2*, *STK11*, *SDHB*, *PRSS1*, *ATM*, *MSR1*, and *PALB2* [8]. Very recently, a whole exome analysis on 54 *CDH1*-negative GC patients did not identify obvious candidates for GC predisposition [9], while, a gene panel-based analysis of 333 HDGC and non-HDGC cases identified 11 mutation carriers of *PALB2*, *BRCA1*, and *RAD51C*, which are genes involved in DNA homologous recombination (HR) [10]. A recent meta-analysis, performed on NGS published data, identified a list of genes carrying deleterious variants in families meeting the 2020 HDGC clinical criteria [11]. Pathogenic or likely pathogenic variants were found in candidate genes involved in DNA damage response pathways [11], encompassing *ATM* [12, 13], *BRCA1* [13], *BRCA2* [8, 13], *PALB2* [8, 10, 13, 14], *RAD51C* [10], and *ATR* [14]. In fact, *PALB2* and *ATM* were the most frequently mutated genes in the HDGC setting [11]. The former has been extensively associated with breast cancer predisposition [15], while the latter has been associated with both breast and gastric cancer susceptibility [16, 17]. Interestingly, *PALB2* loss of function variants have been shown to be enriched in the HDGC setting, compared to the general population [14]. While *PALB2* association with HDGC holds promise, *ATM* pleiotropy prevents a clear association with this disease.

7.3 GAPPS

In 2012, GAPPS, was described as an autosomal dominant syndrome [18]. The key clinical features of GAPPS include fundic gland polyposis (FGP) of the stomach with occasional hyperplastic and adenomatous polyps, focal foveolar-type dysplasia, hyperproliferative aberrant pits and development of adenomas with gastric type dysplasia or intestinal-/mixed-type gastric adenocarcinoma [18, 36, 37]. Current diagnostic criteria are depicted in Table 7.2 [36, 38].

In 2016, linkage analysis on six selected families mapped the gene to the 5q22 chromosomal region. Through Sanger sequencing, point mutations in *APC* promoter 1B, that co-segregated with the disease in all three families, were identified [38, 39]. Therefore, GAPPS is considered a part of a broad phenotypic spectrum of inherited polyposis associated with *APC* germline defects, but with tropism to the stomach (see paragraph "Familial Adenomatous Polyposis"). Since then, 12 additional families were found to harbor *APC* promoter 1B single nucleotide variants (SNVs) [40–43]. Two SNVs were found co-segregating within a family with severer phenotype, but their individual contribution remains unclear [38].

GAPPS phenotypes are diverse among individuals, in the number of polyps, from 30 to hundreds and GC age of onset ranging from 23 to 75 years of age [18, 43]. In fact, third-generation individuals display a much severer phenotype than first-generation obligated carriers [18]. Altogether, these observations suggest incomplete penetrance of *APC* promoter 1B SNVs that may be aggravated by environmental factors and moderate/low penetrance variants. Risk to develop intestinal- or mixed-type GC is 13% (Table 7.1) [18].

Surveillance of GAPPS families includes endoscopic surveillance with biopsies and prophylactic gastrectomy, due to a rapid malignant progression of FGP [18, 40, 43].

Table 7.2 GAPPS clinical criteria for genetic testing

Clinical criteria		Genetic screening
Essential criteria	Body and fundus gastric polyps	*APC* promoter 1B SNVs
	No evidence of colorectal or duodenal polyposis	
	>100 proximal stomach polyps or >30 polyps in a first degree relative GAPPS diagnosed patient	
	Predominantly fundic gland polyposis, which may have dysplasia	
	Relative with dysplastic FGPs or GC	
Supportive criteria	Autosomal dominant inheritance pattern	
	Presence of hyperproliferative aberrant pits, hyperplastic polyps, and gastric-type adenomas	

7.4 FIGC

FIGC is the HDGC counterpart that predisposes to intestinal-type gastric cancer (IGC). Current clinical criteria have been defined by the international gastric cancer linkage consortium (IGCLC) in 1999, depending on the GC incidence in the population and are depicted in Table 7.3 [44, 45]. Countries with a high GC incidence, such as Japan and Portugal, should use criteria analogous to those proposed for Lynch syndrome [46], while in countries with a low GC incidence, including USA and UK, FIGC selection criteria are more restrictive.

To date, no germline defects have been found to be recurrently associated with FIGC predisposition, which currently has unknown age of onset, tumor spectrum, and penetrance. Thus, clinical criteria have not been updated or validated since firstly described in 1999 [44]. Recently, the average IGC age of onset in FIGC families was found to be 10 years earlier than observed for the sporadic setting [19]. At the somatic level, *TP53*, *BRCA2*, *ATM*, *FOXF1*, *FHIT*, *SDHB*, *MSH6*, *CTNNA1*, and *PXN* were found mutated at higher frequencies in tumors from FIGC patients than in sporadic IGC, which also correlates with increased MSI frequency. The FIGC tumor spectrum is broad and predisposes to IGC, but also to colorectal and breast cancer, at lower frequencies [19]. A recent meta-analysis found *BRCA2* as the most frequently mutated gene in the germline DNA of FIGC probands, reaching 17% [11], a frequency that was similar to that of *BRCA2* somatic variants in sporadic IGC (9%) and higher than that of sporadic DGC (5%) [47].

Carvalho and colleagues [19] hint toward FIGC as a polygenic syndrome, since germline defects in major genes were not found in a large FIGC cohort. These authors also proposed redefinition of clinical criteria for FIGC to at least 2 GC cases diagnosed at any age, with one histologically confirmed as IGC [19].

Considering the number of genes that can be involved in this disease, the lifetime GC risk is not easy to determine due to the high genetic variability (Table 7.1).

Current surveillance is evaluated and applied on a case-by-case basis, yet recommendations include endoscopy in first-degree relatives, 10 years earlier than the earliest IGC age of onset [48], or gastroduodenoscopy at 40 years of age or 5 years earlier than the youngest IGC diagnosed in the family [49]. Eradication of *H. pylori* infection is recommended in FIGC families, due to its high frequency in this setting [49].

Table 7.3 FIGC clinical criteria

Clinical criteria		Genetic screening
High GC incidence countries	At least three relatives with IGC, one first-degree of the other two	Unknown germline cause
	At least two successive generations affected	
	GC diagnosed <50 years of age in at least one relative	
Low GC incidence countries	At least two first/second-degree relatives with IGC, one diagnosed <50 years of age	
	At least three relatives with IGC at any age	

7.5 Non-polyposis Syndromes

7.5.1 Lynch Syndrome

Lynch syndrome (LS) predisposes to colorectal and endometrial cancers and follow an autosomal dominant inheritance pattern [50]. LS is caused by pathogenic variants in *MLH1*, *MSH2*, *MSH6*, and *PMS2*, that encode the DNA mismatch repair (MMR) proteins [51], or by large deletions of the *EPCAM* gene, located upstream of *MSH2* [52]. MMR proteins work in a coordinated mode to repair the DNA mismatches that arise during DNA replication and recombination [53].

LS patients also have an increased risk of developing other tumors [54, 55], encompassing a lifetime risk to develop gastric cancer, estimated to be 1–10%, according to the altered gene (Table 7.1).

Regarding GC surveillance, LS patients with an *MLH1*/*MSH2* pathogenic variant, a family history of GC, and other risk factors should undergo upper endoscopy every 3–5 years beginning at age 40 [24].

Moreover, patients with LS, who have a deficiency of the MMR system (dMMR), can benefit from chemoprevention based on the daily use of aspirin [56] and, in case MSI cancers develop, may be treated with anti-PD-1/PD-L1 therapy [57, 58].

7.5.2 Li-Fraumeni Syndrome

The *TP53* gene is located on chromosome 17p13.1 and encodes the p53 protein, a tumor suppressor that responds to different cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or metabolism changes [59]. Due to its crucial function in maintaining the genomic stability, p53 has been defined as "the guardian of the genome" and, indeed, *TP53* somatic alterations are present in approximately 50% of sporadic tumors [60], conferring to p53 an important role as a biomarker for the diagnosis, tumor progression, poor prognosis, and reduced sensitivity for anticancer drugs [61].

Germline pathogenic variants in the *TP53* gene are associated with Li-Fraumeni syndrome (LFS), a rare autosomal dominant disorder characterized by a high predisposition to several types of cancer, such as brain tumors, breast cancer, sarcomas, acute leukemia, and adrenocortical tumors [28, 62–71].

The lifetime risk of GC for patients with LFS, although not consensual, has been estimated to be 2–5% (Table 7.1) [28, 72, 73].

Given the risk of developing gastrointestinal cancers, the guidelines suggest that LFS patients should undergo upper endoscopy and colonoscopy every 2–5 years starting from age 25 years [29]. Moreover, in children, the recommendations are to perform clinical examination and abdominal ultrasound every 6 months, annual whole-body MRI, and brain MRI from the first year of life, if the *TP53* variant is known to be associated with childhood cancers. In adults, the surveillance should include every year clinical examination, whole-body MRI, breast MRI in females from 20 until 65 years, and brain MRI until 50 years [63].

7.5.3 BRCA1- and BRCA2-Associated Hereditary Breast and Ovarian Cancer

The *BRCA1* gene, located on chromosome 17q21.31, encodes a nuclear protein involved in DNA repair, cell cycle checkpoint control, and maintenance of genomic stability forming a large multi-subunit protein complex known as BRCA1-associated genome surveillance complex (BASC) [74–77].

The *BRCA2* gene is located on chromosome 13q13.1 and encodes a nuclear protein involved in repairing damaged DNA, recruiting the recombinase RAD51 to the DNA double-strand breaks (DSBs) through the formation of a BRCA1-PALB2-BRCA2 complex [74, 75, 78, 79].

Germline pathogenic variants in *BRCA1* and *BRCA2* genes are associated with the hereditary breast and ovarian cancer (HBOC) syndrome [80], characterized by a high risk of developing breast and ovarian cancer in females [81–83], breast and prostate cancer in males [84–87] and pancreatic cancer in both sexes [88–90].

Further, *BRCA1* pathogenic variants have been associated with an increased risk of colon cancer [91] and *BRCA2* pathogenic variants have been associated with uveal melanoma [92, 93].

Recently, pathogenic variants in *BRCA1/2* and other genes involved in breast/ovarian cancer predisposition have been associated with an increased GC risk [8, 10, 12–14]. The IGC risk is estimated to be 2% in *BRCA1/2* pathogenic variant carriers (Table 7.1) [30], however prevention should be evaluated on the basis of family history [24].

Moreover, the discovery of the therapeutic potential of inhibitors of the poly adenosine-diphosphate ribose polymerase (PARP) in carriers of germline/somatic *BRCA1/2* pathogenic variants with ovarian, breast, prostate, and pancreatic cancers led to a revolution in the treatment of these tumors [94–100]. PARP inhibitors have shown their efficacy also in patients with pathogenic variants in genes involved in the HR pathway [101–104]. These results pave the way for the future use of PARP inhibitors in all tumors with a deficiency of the HR system, independently of the germline or somatic nature of the alteration, including GC [105].

7.6 Polyposis Syndromes

7.6.1 Familial Adenomatous Polyposis

The APC protein is a tumor suppressor that acts as a Wnt signaling antagonist, and regulates transcriptional activation, cell migration and apoptosis [106]. Pathogenic or likely pathogenic alterations in the *APC* gene (chromosome 5q22.2) predispose to familial adenomatous polyposis (FAP) [107, 108]. This autosomal dominant syndrome is characterized by polyposis and carcinomas in the gastrointestinal tract, as well as, extra-gastrointestinal carcinomas, such as thyroid [34]. While classical FAP predisposes to hundreds to thousands of colonic and rectal polyps that may develop into colorectal carcinoma, attenuated FAP (AFAP) displays a much milder

phenotype [34, 109]. Families with AFAP present fewer and latter-onset of both polyps and carcinomas, as well as cancer-decreased risk [110]. The phenotype severity is dependent on the mutation location within the *APC* gene [111], as above mentioned for GAPPS with unique predisposition to the stomach [38].

FAP and AFAP also predispose to gastric polyps in >60% and 93% of patients, respectively [112]. However, gastric adenocarcinoma risk ranges between 4% and 7% in the Asian population, with no increased risk for the western population (Table 7.1) [24, 31]. In fact, FGP and focal low-grade dysplasia in the stomach commonly do not undergo malignant transformation [113, 114]. Nevertheless, increased risk is observed in the presence of FGP stomach carpeting, polyps larger than 20 mm, tubular adenomas, high-grade dysplasia polyps, pyloric gland adenomas, and in specific geographical areas [31, 115, 116]. According to these high-risk features and family history, specialized surveillance or gastrectomy may be recommended [24].

7.6.2 MUTYH-Associated Polyposis

The *MUTYH* gene is located on chromosome 1p34.1 and encodes the MutY DNA glycosylase, involved in oxidative DNA damage repair and, if unrepaired, apoptosis signaling [117].

MUTYH-associated polyposis (MAP) distinguishes from (A)FAP by presenting a recessive inheritance pattern with reduced risk for colonic and duodenal adenomas (fewer than 100) and carcinomas (5%). Thus, biallelic pathogenic or likely pathogenic variants in *MUTYH* (chromosome 1p34.1) predispose to MAP [118]. Risk to develop IGC ranges from 2% for females to 4% for males (Table 7.1) [32].

Current surveillance measurements include upper endoscopy and side viewing duodenoscopy every 3 months to 4 years beginning at age 30–35 years with subsequent follow-up based on initial findings [24, 119, 120].

7.6.3 Juvenile Polyposis Syndrome

The *BMPR1A* gene, located on chromosome 10q23.2, encodes the bone morphogenetic protein receptor type IA, a transmembrane serine/threonine kinase that binds members of the TGF-β superfamily and plays a role in signal transduction, apoptosis and cell differentiation [121].

The *SMAD4* gene (chromosome 18q21.2) encodes a member of the Smad family of signal transduction proteins that are activated by transmembrane serine-threonine receptor kinases in response to TGF-β and bone morphogenetic protein signaling pathways. *SMAD4* is a transcription factor that acts as a tumor suppressor and inhibits epithelial cell proliferation [122].

Germline pathogenic variants in *BMPR1A* and *SMAD4* genes are associated with juvenile polyposis syndrome (JPS), an autosomal dominant disorder, that

predisposes to hamartomatous polyps in the gastrointestinal tract, specifically in the stomach, small intestine, colon, and rectum [123].

The majority of juvenile polyps are benign, however can undergo malignant transformation. Lifetime estimates of developing gastrointestinal cancers in families with JPS range from 11% to 86%, with variability by region, time period included, and associated gene [124–128]. In fact, approximately 15% of JPS individuals develop cancer [127, 129]. While, the GC incidence is approximately around 10–30% in JPS patients with gastric polyps (Table 7.1) [130, 131], the risk of colorectal cancer ranges between 17% and 22% by 35 years of age and approaches 68% by 60 years of age [132]. In JPS context, small bowel and pancreatic cancers have also been reported [133–137]. Individuals with *SMAD4*-related JPS are more likely to have a personal or family history of upper gastrointestinal polyps than individuals with a *BMPR1A* pathogenic variant. The gastric phenotype in individuals with a *SMAD4* pathogenic variant tends to be more aggressive with significant polyposis, anemia, and a higher GC risk [125, 127, 128].

According to the clinical practice guidelines for JPS, the gastric surveillance recommended for individuals with a *BMPR1A* or *SMAD4* pathogenic variant includes colonoscopy and upper endoscopy every 3 years beginning at age 15 or earlier if symptomatic. If polyps are found, after polyp treatment an annual screening is recommended until no polyps are found, followed by a screening every 3 years [24, 138, 139].

7.6.4 Peutz-Jeghers Syndrome

The *STK11* gene (formerly *LKB1*) is located on chromosome 19p13.3 and encodes a serine/threonine kinase that acts as a tumor suppressor, regulating energy metabolism and cell polarity [140].

Germline pathogenic variants in the *STK11* gene are associated with Peutz-Jeghers syndrome (PJS), an autosomal dominant syndrome. PJS is characterized by melanocytic macules of the lips, buccal mucosa and digits, multiple gastrointestinal hamartomatous polyps, and an increased risk for different tumors, encompassing colorectal, gastric, pancreatic, breast, and ovarian cancers [141].

In *STK11* pathogenic variant carriers, the lifetime GC risk is estimated to be 29% (Table 7.1) [33, 34, 142, 143]. For this reason, the clinical guidelines suggest that PJS patients should undergo upper endoscopy with polypectomy every 2–3 years, starting at the age of 18; shorter intervals may be indicated based on polyp size, number, and pathology [24].

7.6.5 PTEN Hamartoma Tumor Syndrome

The *PTEN* gene (chromosome 10q23.31) encodes a phosphatase which antagonizes the PI3K signaling pathway and negatively regulates the MAPK pathway [144].

Germline pathogenic variants in *PTEN* are associated with the PTEN hamartoma tumor syndrome (PHTS) that includes Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, PTEN-related Proteus syndrome, and PTEN-related proteus-like syndrome [145].

Cowden syndrome (CS) is an autosomal dominant disorder that predisposes to benign hamartomas and increased lifetime risk of breast, thyroid, uterine, colorectal, and other cancers, including stomach [145–147]. Upper or lower gastrointestinal polyps occur in more than 90% of individuals with a *PTEN* pathogenic variant [148]. In the stomach, the most common findings are hyperplastic polyps, hamartomas, and ganglioneuromas [149–151].

Cowden syndrome does not have increased risk of gastric malignancy (Table 7.1); however, complications of benign neoplasm can occur [35]. Indeed, some CS patients have symptoms including hemorrhage, obstruction, and pain [35]. According to the guidelines, PHTS patients should undergo upper and lower endoscopy with removal of polyps beginning at age 35 years with frequency dependent on degree of polyposis identified [145].

7.7 Conclusions

GC is one of the most common and deadly tumors and, among risk factors for the development of this cancer, genetic predisposition plays an important role.

Besides HDGC, associated with *CDH1* and *CTNNA1* pathogenic variants, other genetic syndromes characterized by high risk to develop GC have been described: GAPPS, associated with genetic variants in the *APC* promoter, and FIGC, still lacking a clear genetic cause.

In addition to these three syndromes, genes including *TP53*, *BRCA1/2*, and MMR genes, whose variants are associated with other cancer genetic syndromes, also include an increased risk for GC (Table 7.1).

Moreover, genes associated with the development of gastrointestinal polyps, such as *APC*, *MUTYH*, *BMPR1A*, *SMAD4*, *STK1*, and *PTEN* may also evolve in GC (Table 7.1).

The evidence of GC risk associated with these syndromes and the availability of recommendations for the management of variant carriers suggest that these genes should be included in a gene panel for the identification of patients at risk of developing GC.

In summary, new genes are constantly emerging from NGS studies, showing that GC predisposition is distributed over several genes, with only a small portion of genes being recurrently mutated.

These findings address the choice of wide panels, including the genes involved in the main cancer syndromes. This creates new diagnostic opportunities but also increases the risk of an incorrect genetic diagnosis [152]. Importantly, the identification of a pathogenic germline variant can not only guide the choice of the best chemoprevention and prophylactic surgeries but also the choice of novel targeted therapies, toward personalized medicine based on the genetic characteristics of each patient.

References

1. van der Post RS, Vogelaar IP, Carneiro F et al (2015) Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. J Med Genet 52:361–374. https://doi.org/10.1136/jmedgenet-2015-103094
2. Guilford P, Hopkins J, Harraway J et al (1998) E-cadherin germline mutations in familial gastric cancer. Nature 392:402–405. https://doi.org/10.1038/32918
3. Majewski IJ, Kluijt I, Cats A et al (2013) An α-E-catenin (CTNNA1) mutation in hereditary diffuse gastric cancer. J Pathol 229:621–629. https://doi.org/10.1002/path.4152
4. Weren RDA, van der Post RS, Vogelaar IP et al (2018) Role of germline aberrations affecting CTNNA1, MAP3K6 and MYD88 in gastric cancer susceptibility. J Med Genet 55:669–674. https://doi.org/10.1136/jmedgenet-2017-104962
5. Lobo S, Benusiglio PR, Coulet F et al (2021) Cancer predisposition and germline CTNNA1 variants. Eur J Med Genet 64:104316. https://doi.org/10.1016/j.ejmg.2021.104316
6. Gaston D, Hansford S, Oliveira C et al (2014) Germline mutations in MAP3K6 are associated with familial gastric cancer. PLoS Genet 10:e1004669. https://doi.org/10.1371/journal.pgen.1004669
7. Vogelaar IP, Ligtenberg MJL, van der Post RS et al (2016) Recurrent candidiasis and early-onset gastric cancer in a patient with a genetically defined partial MYD88 defect. Familial Cancer 15:289–296. https://doi.org/10.1007/s10689-015-9859-z
8. Hansford S, Kaurah P, Li-Chang H et al (2015) Hereditary diffuse gastric cancer syndrome: CDH1 mutations and beyond. JAMA Oncol 1:23–32. https://doi.org/10.1001/jamaoncol.2014.168
9. Vogelaar IP, van der Post RS, van Krieken JHJ et al (2017) Unraveling genetic predisposition to familial or early onset gastric cancer using germline whole-exome sequencing. Eur J Hum Genet 25:1246–1252. https://doi.org/10.1038/ejhg.2017.138
10. Sahasrabudhe R, Lott P, Bohorquez M et al (2017) Germline mutations in PALB2, BRCA1, and RAD51C, which regulate DNA recombination repair, in patients with gastric cancer. Gastroenterology 152:983–986.e6. https://doi.org/10.1053/j.gastro.2016.12.010
11. Garcia-Pelaez J, Barbosa-Matos R, São José C et al (2022) Gastric cancer genetic predisposition and clinical presentations: established heritable causes and potential candidate genes. Eur J Med Genet 65:104401. https://doi.org/10.1016/j.ejmg.2021.104401
12. Slavin T, Neuhausen SL, Rybak C et al (2017) Genetic gastric cancer susceptibility in the international clinical cancer genomics community research network. Cancer Genet 216–217:111–119. https://doi.org/10.1016/j.cancergen.2017.08.001
13. Tedaldi G, Pirini F, Tebaldi M et al (2019) Multigene panel testing increases the number of loci associated with gastric cancer predisposition. Cancers (Basel) 11. https://doi.org/10.3390/cancers11091340
14. Fewings E, Larionov A, Redman J et al (2018) Germline pathogenic variants in PALB2 and other cancer-predisposing genes in families with hereditary diffuse gastric cancer without CDH1 mutation: a whole-exome sequencing study. Lancet Gastroenterol Hepatol 3:489–498. https://doi.org/10.1016/S2468-1253(18)30079-7
15. Antoniou AC, Casadei S, Heikkinen T et al (2014) Breast-cancer risk in families with mutations in PALB2. N Engl J Med 371:497–506. https://doi.org/10.1056/NEJMoa1400382
16. Helgason H, Rafnar T, Olafsdottir HS et al (2015) Loss-of-function variants in ATM confer risk of gastric cancer. Nat Genet 47:906–910. https://doi.org/10.1038/ng.3342
17. Moslemi M, Moradi Y, Dehghanbanadaki H et al (2021) The association between ATM variants and risk of breast cancer: a systematic review and meta-analysis. BMC Cancer 21:27. https://doi.org/10.1186/s12885-020-07749-6
18. Worthley DL, Phillips KD, Wayte N et al (2012) Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS): a new autosomal dominant syndrome. Gut 61:774–779. https://doi.org/10.1136/gutjnl-2011-300348

19. Carvalho J, Oliveira P, Senz J et al (2020) Redefinition of familial intestinal gastric cancer: clinical and genetic perspectives. J Med Genet 58:1. https://doi.org/10.1136/jmedgenet-2019-106346
20. Cheng L, Eng C, Nieman LZ et al (2011) Trends in colorectal cancer incidence by anatomic site and disease stage in the United States from 1976 to 2005. Am J Clin Oncol 34:573–580. https://doi.org/10.1097/COC.0b013e3181fe41ed
21. Siegel RL, Medhanie GA, Fedewa SA, Jemal A (2019) State variation in early-onset colorectal cancer in the United States, 1995-2015. J Natl Cancer Inst 111:1104–1106. https://doi.org/10.1093/jnci/djz098
22. Giardiello FM, Offerhaus JG Phenotype and cancer risk of various polyposis syndromes. Eur J Cancer 31A:1085–7. https://doi.org/10.1016/0959-8049(95)00139-a
23. Møller P, Seppälä TT, Bernstein I et al (2018) Cancer risk and survival in path_MMR carriers by gene and gender up to 75 years of age: a report from the prospective Lynch syndrome database. Gut 67:1306–1316. https://doi.org/10.1136/gutjnl-2017-314057
24. National Comprehensive Cancer Network (NCCN) (2022) Genetic/Familial High-Risk Assessment: Colorectal v 2.2021. https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf
25. Siegel RL, Miller KD, Fuchs HE, Jemal A (2022) Cancer statistics, 2022. CA Cancer J Clin 72:7–33. https://doi.org/10.3322/caac.21708
26. Bailey CE, Hu C-Y, You YN et al (2015) Increasing disparities in the age-related incidences of colon and rectal cancers in the United States, 1975-2010. JAMA Surg 150:17–22. https://doi.org/10.1001/jamasurg.2014.1756
27. Rumilla K, Schowalter KV, Lindor NM et al (2011) Frequency of deletions of EPCAM (TACSTD1) in MSH2-associated Lynch syndrome cases. J Mol Diagn 13:93–99. https://doi.org/10.1016/j.jmoldx.2010.11.011
28. Masciari S, Dewanwala A, Stoffel EM et al (2011) Gastric cancer in individuals with Li-Fraumeni syndrome. Genet Med 13:651–657. https://doi.org/10.1097/GIM.0b013e31821628b6
29. National Comprehensive Cancer Network (NCCN) (2022) Genetic/familial high-risk assessment: breast, ovarian, and pancreatic v. 2.2022. https://www.nccn.org/professionals/physician_gls/pdf/genetics_bop.pdf
30. Friedenson B (2005) BRCA1 and BRCA2 pathways and the risk of cancers other than breast or ovarian. MedGenMed 7:60
31. Shibata C, Ogawa H, Miura K et al (2013) Clinical characteristics of gastric cancer in patients with familial adenomatous polyposis. Tohoku J Exp Med 229:143–146. https://doi.org/10.1620/tjem.229.143
32. Win AK, Reece JC, Dowty JG et al (2016) Risk of extracolonic cancers for people with biallelic and monoallelic mutations in MUTYH. Int J Cancer 139:1557–1563. https://doi.org/10.1002/ijc.30197
33. Hearle N, Schumacher V, Menko FH et al (2006) Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. Clin Cancer Res 12:3209–3215. https://doi.org/10.1158/1078-0432.CCR-06-0083
34. Syngal S, Brand RE, Church JM et al (2015) ACG clinical guideline: genetic testing and management of hereditary gastrointestinal cancer syndromes. Am J Gastroenterol 110:223–262. https://doi.org/10.1038/ajg.2014.435
35. Correia TF, Mesquita I, Marcos M, et al (2021) Surgical approach to gastric polyposis in Cowen syndrome-case report. J Surg Case Rep 2021:rjab258. https://doi.org/10.1093/jscr/rjab258
36. Rudloff U (2018) Gastric adenocarcinoma and proximal polyposis of the stomach: diagnosis and clinical perspectives. Clin Exp Gastroenterol 11:447–459. https://doi.org/10.2147/CEG.S163227
37. Gullo I, van der Post RS, Carneiro F (2021) Recent advances in the pathology of heritable gastric cancer syndromes. Histopathology 78:125–147. https://doi.org/10.1111/his.14228

38. Li J, Woods SL, Healey S et al (2016) Point mutations in exon 1B of APC reveal gastric adenocarcinoma and proximal polyposis of the stomach as a familial adenomatous polyposis variant. Am J Hum Genet 98:830–842. https://doi.org/10.1016/j.ajhg.2016.03.001
39. Beer A, Streubel B, Asari R et al (2017) Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) - a rare recently described gastric polyposis syndrome - report of a case. Z Gastroenterol 55:1131–1134. https://doi.org/10.1055/s-0043-117182
40. Foretová L, Navrátilová M, Svoboda M et al (2019) GAPPS - gastric adenocarcinoma and proximal polyposis of the stomach syndrome in 8 families tested at Masaryk Memorial Cancer Institute - prevention and prophylactic gastrectomies. Klin Onkol 32:109–117. https://doi.org/10.14735/amko2019S109
41. Kanemitsu K, Iwatsuki M, Yamashita K et al (2021) Two Asian families with gastric adenocarcinoma and proximal polyposis of the stomach successfully treated via laparoscopic total gastrectomy. Clin J Gastroenterol 14:92–97. https://doi.org/10.1007/s12328-020-01290-6
42. Mitsui Y, Yokoyama R, Fujimoto S et al (2018) First report of an Asian family with gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) revealed with the germline mutation of the APC exon 1B promoter region. Gastric Cancer 21:1058–1063. https://doi.org/10.1007/s10120-018-0855-5
43. Repak R, Kohoutova D, Podhola M et al (2016) The first European family with gastric adenocarcinoma and proximal polyposis of the stomach: case report and review of the literature. Gastrointest Endosc 84:718–725. https://doi.org/10.1016/j.gie.2016.06.023
44. Caldas C, Carneiro F, Lynch HT et al (1999) Familial gastric cancer: overview and guidelines for management. J Med Genet 36:873–880
45. Oliveira C, Suriano G, Ferreira P et al (2004) Genetic screening for familial gastric cancer. Hered Cancer Clin Pract 2:51–64. https://doi.org/10.1186/1897-4287-2-2-51
46. Shinmura K, Kohno T, Takahashi M et al (1999) Familial gastric cancer: clinicopathological characteristics, RER phenotype and germline p53 and E-cadherin mutations. Carcinogenesis 20:1127–1131. https://doi.org/10.1093/carcin/20.6.1127
47. Garcia-Pelaez J, Barbosa-Matos R, Gullo I et al (2021) Histological and mutational profile of diffuse gastric cancer: current knowledge and future challenges. Mol Oncol 1–27:2841. https://doi.org/10.1002/1878-0261.12948
48. Sereno M, Aguayo C, Guillén Ponce C et al (2011) Gastric tumours in hereditary cancer syndromes: clinical features, molecular biology and strategies for prevention. Clin Transl Oncol 13:599–610. https://doi.org/10.1007/s12094-011-0705-y
49. Corso G, Roncalli F, Marrelli D et al (2013) History, pathogenesis, and management of familial gastric cancer: original study of John XXIII's family. Biomed Res Int 2013:385132. https://doi.org/10.1155/2013/385132
50. Kohlmann W, Gruber SB (2004) Lynch syndrome. GeneReviews® (updated 2021)
51. Chung DC, Rustgi AK (1995) DNA mismatch repair and cancer. Gastroenterology 109:1685–1699. https://doi.org/10.1016/0016-5085(95)90660-6
52. Tutlewska K, Lubinski J, Kurzawski G (2013) Germline deletions in the EPCAM gene as a cause of Lynch syndrome - literature review. Hered Cancer Clin Pract 11:9. https://doi.org/10.1186/1897-4287-11-9
53. Iyer RR, Pluciennik A, Burdett V, Modrich PL (2006) DNA mismatch repair: functions and mechanisms. Chem Rev 106:302–323. https://doi.org/10.1021/cr0404794
54. Bonadona V, Bonaïti B, Olschwang S et al (2011) Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. JAMA 305:2304–2310. https://doi.org/10.1001/jama.2011.743
55. Walsh MD, Buchanan DD, Cummings MC et al (2010) Lynch syndrome-associated breast cancers: clinicopathologic characteristics of a case series from the colon cancer family registry. Clin Cancer Res 16:2214–2224. https://doi.org/10.1158/1078-0432.CCR-09-3058
56. Burn J, Bishop DT, Mecklin J-P et al (2008) Effect of aspirin or resistant starch on colorectal neoplasia in the Lynch syndrome. N Engl J Med 359:2567–2578. https://doi.org/10.1056/NEJMoa0801297

57. Le DT, Uram JN, Wang H et al (2015) PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 372:2509–2520. https://doi.org/10.1056/NEJMoa1500596
58. Zhao P, Li L, Jiang X, Li Q (2019) Mismatch repair deficiency/microsatellite instability-high as a predictor for anti-PD-1/PD-L1 immunotherapy efficacy. J Hematol Oncol 12:54. https://doi.org/10.1186/s13045-019-0738-1
59. Harris CC (1996) Structure and function of the p53 tumor suppressor gene: clues for rational cancer therapeutic strategies. J Natl Cancer Inst 88:1442–1455. https://doi.org/10.1093/jnci/88.20.1442
60. Ozaki T, Nakagawara A (2011) p53: the attractive tumor suppressor in the cancer research field. J Biomed Biotechnol 2011:603925. https://doi.org/10.1155/2011/603925
61. Boyd MT, Vlatkovic N (2008) p53: a molecular marker for the detection of cancer. Expert Opin Med Diagn 2:1013–1024. https://doi.org/10.1517/17530059.2.9.1013
62. Schneider K, Zelley K, Nichols KE, Garber J (1999) Li-Fraumeni Syndrome. GeneReviews® (updated 2019)
63. Frebourg T, Bajalica Lagercrantz S, Oliveira C et al (2020) Guidelines for the Li-Fraumeni and heritable TP53-related cancer syndromes. Eur J Hum Genet 28:1379–1386. https://doi.org/10.1038/s41431-020-0638-4
64. Li FP, Fraumeni JF (1969) Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? Ann Intern Med 71:747–752. https://doi.org/10.7326/0003-4819-71-4-747
65. Chompret A, Brugières L, Ronsin M et al (2000) P53 germline mutations in childhood cancers and cancer risk for carrier individuals. Br J Cancer 82:1932–1937. https://doi.org/10.1054/bjoc.2000.1167
66. Nichols KE, Malkin D, Garber JE et al (2001) Germ-line p53 mutations predispose to a wide spectrum of early-onset cancers. Cancer Epidemiol Biomark Prev 10:83–87
67. Olivier M, Goldgar DE, Sodha N et al (2003) Li-Fraumeni and related syndromes: correlation between tumor type, family structure, and TP53 genotype. Cancer Res 63:6643–6650
68. Varley JM (2003) Germline TP53 mutations and Li-Fraumeni syndrome. Hum Mutat 21:313–320. https://doi.org/10.1002/humu.10185
69. Gonzalez KD, Noltner KA, Buzin CH et al (2009) Beyond Li Fraumeni syndrome: clinical characteristics of families with p53 germline mutations. J Clin Oncol 27:1250–1256. https://doi.org/10.1200/JCO.2008.16.6959
70. Wong P, Verselis SJ, Garber JE et al (2006) Prevalence of early onset colorectal cancer in 397 patients with classic Li-Fraumeni syndrome. Gastroenterology 130:73–79. https://doi.org/10.1053/j.gastro.2005.10.014
71. Birch JM, Alston RD, McNally RJ et al (2001) Relative frequency and morphology of cancers in carriers of germline TP53 mutations. Oncogene 20:4621–4628. https://doi.org/10.1038/sj.onc.1204621
72. Bougeard G, Renaux-Petel M, Flaman JM et al (2015) Revisiting Li-Fraumeni syndrome from TP53 mutation carriers. J Clin Oncol 33:2345–2352. https://doi.org/10.1200/JCO.2014.59.5728
73. Mai PL, Best AF, Peters JA et al (2016) Risks of first and subsequent cancers among TP53 mutation carriers in the National Cancer Institute Li-Fraumeni syndrome cohort. Cancer 122:3673–3681. https://doi.org/10.1002/cncr.30248
74. Roy R, Chun J, Powell SN (2011) BRCA1 and BRCA2: different roles in a common pathway of genome protection. Nat Rev Cancer 12:68–78. https://doi.org/10.1038/nrc3181
75. Foulkes WD, Shuen AY (2013) In brief: BRCA1 and BRCA2. J Pathol 230:347–349. https://doi.org/10.1002/path.4205
76. Wang Y, Cortez D, Yazdi P et al (2000) BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. Genes Dev 14:927–939
77. Hedenfalk IA, Ringnér M, Trent JM, Borg A (2002) Gene expression in inherited breast cancer. Adv Cancer Res 84:1–34

78. Xia B, Sheng Q, Nakanishi K et al (2006) Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2. Mol Cell 22:719–729. https://doi.org/10.1016/j.molcel.2006.05.022
79. Buisson R, Dion-Côté A-M, Coulombe Y et al (2010) Cooperation of breast cancer proteins PALB2 and piccolo BRCA2 in stimulating homologous recombination. Nat Struct Mol Biol 17:1247–1254. https://doi.org/10.1038/nsmb.1915
80. Petrucelli N, Daly MB, Pal T (1998) BRCA1- and BRCA2-associated hereditary breast and ovarian cancer. GeneReviews® (updated 2022)
81. Antoniou A, Pharoah PDP, Narod S et al (2003) Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 72:1117–1130. https://doi.org/10.1086/375033
82. Chen S, Parmigiani G (2007) Meta-analysis of BRCA1 and BRCA2 penetrance. J Clin Oncol 25:1329–1333. https://doi.org/10.1200/JCO.2006.09.1066
83. Mavaddat N, Peock S, Frost D et al (2013) Cancer risks for BRCA1 and BRCA2 mutation carriers: results from prospective analysis of EMBRACE. J Natl Cancer Inst 105:812–822. https://doi.org/10.1093/jnci/djt095
84. Tai YC, Domchek S, Parmigiani G, Chen S (2007) Breast cancer risk among male BRCA1 and BRCA2 mutation carriers. J Natl Cancer Inst 99:1811–1814. https://doi.org/10.1093/jnci/djm203
85. Leongamornlert D, Mahmud N, Tymrakiewicz M et al (2012) Germline BRCA1 mutations increase prostate cancer risk. Br J Cancer 106:1697–1701. https://doi.org/10.1038/bjc.2012.146
86. Kote-Jarai Z, Leongamornlert D, Saunders E et al (2011) BRCA2 is a moderate penetrance gene contributing to young-onset prostate cancer: implications for genetic testing in prostate cancer patients. Br J Cancer 105:1230–1234. https://doi.org/10.1038/bjc.2011.383
87. Tedaldi G, Tebaldi M, Zampiga V et al (2020) Male breast cancer: results of the application of multigene panel testing to an Italian cohort of patients. Diagnostics (Basel, Switzerland) 10. https://doi.org/10.3390/diagnostics10050269
88. Thompson D, Easton DF, Breast Cancer Linkage Consortium (2002) Cancer incidence in BRCA1 mutation carriers. J Natl Cancer Inst 94:1358–1365. https://doi.org/10.1093/jnci/94.18.1358
89. Ghiorzo P, Pensotti V, Fornarini G et al (2012) Contribution of germline mutations in the BRCA and PALB2 genes to pancreatic cancer in Italy. Familial Cancer 11:41–47. https://doi.org/10.1007/s10689-011-9483-5
90. Iqbal J, Ragone A, Lubinski J et al (2012) The incidence of pancreatic cancer in BRCA1 and BRCA2 mutation carriers. Br J Cancer 107:2005–2009. https://doi.org/10.1038/bjc.2012.483
91. Ford D, Easton DF, Bishop DT et al (1994) Risks of cancer in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. Lancet 343:692–695. https://doi.org/10.1016/s0140-6736(94)91578-4
92. Moran A, O'Hara C, Khan S et al (2012) Risk of cancer other than breast or ovarian in individuals with BRCA1 and BRCA2 mutations. Familial Cancer 11:235–242. https://doi.org/10.1007/s10689-011-9506-2
93. Breast Cancer Linkage Consortium (1999) Cancer risks in BRCA2 mutation carriers. J Natl Cancer Inst 91:1310–1316. https://doi.org/10.1093/jnci/91.15.1310
94. Fong PC, Boss DS, Yap TA et al (2009) Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med 361:123–134. https://doi.org/10.1056/NEJMoa0900212
95. Sandhu SK, Schelman WR, Wilding G et al (2013) The poly(ADP-ribose) polymerase inhibitor niraparib (MK4827) in BRCA mutation carriers and patients with sporadic cancer: a phase 1 dose-escalation trial. Lancet Oncol 14:882–892. https://doi.org/10.1016/S1470-2045(13)70240-7

96. Cortesi L, Rugo HS, Jackisch C (2021) An overview of PARP inhibitors for the treatment of breast cancer. Target Oncol 16:255. https://doi.org/10.1007/s11523-021-00796-4
97. Audeh MW, Carmichael J, Penson RT et al (2010) Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. Lancet 376:245–251. https://doi.org/10.1016/S0140-6736(10)60893-8
98. Tutt A, Robson M, Garber JE et al (2010) Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. Lancet (London, England) 376:235–244. https://doi.org/10.1016/S0140-6736(10)60892-6
99. Sandhu SK, Omlin A, Hylands L et al (2013) Poly (ADP-ribose) polymerase (PARP) inhibitors for the treatment of advanced germline BRCA2 mutant prostate cancer. Ann Oncol 24:1416–1418. https://doi.org/10.1093/annonc/mdt074
100. Golan T, Hammel P, Reni M et al (2019) Maintenance Olaparib for germline BRCA-mutated metastatic pancreatic cancer. N Engl J Med 381:317–327. https://doi.org/10.1056/NEJMoa1903387
101. Hodgson DR, Dougherty BA, Lai Z et al (2018) Candidate biomarkers of PARP inhibitor sensitivity in ovarian cancer beyond the BRCA genes. Br J Cancer 119:1401–1409. https://doi.org/10.1038/s41416-018-0274-8
102. Li A, Geyer FC, Blecua P et al (2019) Homologous recombination DNA repair defects in PALB2-associated breast cancers. NPJ Breast Cancer 5:23. https://doi.org/10.1038/s41523-019-0115-9
103. Konstantinopoulos PA, Spentzos D, Karlan BY et al (2010) Gene expression profile of BRCAness that correlates with responsiveness to chemotherapy and with outcome in patients with epithelial ovarian cancer. J Clin Oncol 28:3555–3561. https://doi.org/10.1200/JCO.2009.27.5719
104. Mateo J, Lord CJ, Serra V et al (2019) A decade of clinical development of PARP inhibitors in perspective. Ann Oncol 30:1437. https://doi.org/10.1093/annonc/mdz192
105. Wang Y, Zheng K, Huang Y et al (2021) PARP inhibitors in gastric cancer: beacon of hope. J Exp Clin Cancer Res 40:211. https://doi.org/10.1186/s13046-021-02005-6
106. Ilyas M, Tomlinson IP (1997) The interactions of APC, E-cadherin and beta-catenin in tumour development and progression. J Pathol 182:128–137. https://doi.org/10.1002/(SICI)1096-9896(199706)182:2<128::AID-PATH839>3.0.CO;2-Q
107. Dinarvand P, Davaro EP, Doan JV et al (2019) Familial adenomatous polyposis syndrome: an update and review of extraintestinal manifestations. Arch Pathol Lab Med 143:1382–1398. https://doi.org/10.5858/arpa.2018-0570-RA
108. Jasperson KW, Patel SG, Ahnen DJ (1998) APC-associated polyposis conditions. GeneReviews® (updated 2022)
109. Ma H, Brosens LAA, Offerhaus GJA et al (2018) Pathology and genetics of hereditary colorectal cancer. Pathology 50:49–59. https://doi.org/10.1016/j.pathol.2017.09.004
110. Knudsen AL, Bülow S, Tomlinson I et al (2010) Attenuated familial adenomatous polyposis: results from an international collaborative study. Color Dis 12:e243–e249. https://doi.org/10.1111/j.1463-1318.2010.02218.x
111. Nieuwenhuis MH, Vasen HFA (2007) Correlations between mutation site in APC and phenotype of familial adenomatous polyposis (FAP): a review of the literature. Crit Rev Oncol Hematol 61:153–161. https://doi.org/10.1016/j.critrevonc.2006.07.004
112. Lynch HT, Smyrk T, McGinn T et al (1995) Attenuated familial adenomatous polyposis (AFAP). A phenotypically and genotypically distinctive variant of FAP. Cancer 76:2427–2433. https://doi.org/10.1002/1097-0142(19951215)76:12<2427::aid-cncr2820761205>3.0.co;2-b
113. Garrean S, Hering J, Saied A et al (2008) Gastric adenocarcinoma arising from fundic gland polyps in a patient with familial adenomatous polyposis syndrome. Am Surg 74:79–83

114. Arnason T, Liang W-Y, Alfaro E et al (2014) Morphology and natural history of familial adenomatous polyposis-associated dysplastic fundic gland polyps. Histopathology 65:353–362. https://doi.org/10.1111/his.12393
115. Leone PJ, Mankaney G, Sarvapelli S et al (2019) Endoscopic and histologic features associated with gastric cancer in familial adenomatous polyposis. Gastrointest Endosc 89:961–968. https://doi.org/10.1016/j.gie.2018.12.018
116. Mankaney G, Leone P, Cruise M et al (2017) Gastric cancer in FAP: a concerning rise in incidence. Familial Cancer 16:371–376. https://doi.org/10.1007/s10689-017-9971-3
117. Slupska MM, Baikalov C, Luther WM et al (1996) Cloning and sequencing a human homolog (hMYH) of the Escherichia coli mutY gene whose function is required for the repair of oxidative DNA damage. J Bacteriol 178:3885–3892. https://doi.org/10.1128/jb.178.13.3885-3892.1996
118. Nielsen M, Infante E, Brand R (2012) MUTYH Polyposis. GeneReviews® (updated 2021)
119. Hurley JJ, Thomas LE, Walton S-J et al (2018) The impact of chromoendoscopy for surveillance of the duodenum in patients with MUTYH-associated polyposis and familial adenomatous polyposis. Gastrointest Endosc 88:665–673. https://doi.org/10.1016/j.gie.2018.04.2347
120. Spigelman AD, Williams CB, Talbot IC et al (1989) Upper gastrointestinal cancer in patients with familial adenomatous polyposis. Lancet (London, England) 2:783–785. https://doi.org/10.1016/s0140-6736(89)90840-4
121. Mishina Y, Starbuck MW, Gentile MA et al (2004) Bone morphogenetic protein type IA receptor signaling regulates postnatal osteoblast function and bone remodeling. J Biol Chem 279:27560–27566. https://doi.org/10.1074/jbc.M404222200
122. Massagué J (2012) TGFβ signalling in context. Nat Rev Mol Cell Biol 13:616–630. https://doi.org/10.1038/nrm3434
123. Larsen Haidle J, Howe JR (2003) Juvenile polyposis syndrome. GeneReviews® (updated 2022)
124. Latchford AR, Neale K, Phillips RKS, Clark SK (2012) Juvenile polyposis syndrome: a study of genotype, phenotype, and long-term outcome. Dis Colon Rectum 55:1038–1043. https://doi.org/10.1097/DCR.0b013e31826278b3
125. Aytac E, Sulu B, Heald B et al (2015) Genotype-defined cancer risk in juvenile polyposis syndrome. Br J Surg 102:114–118. https://doi.org/10.1002/bjs.9693
126. Ishida H, Ishibashi K, Iwama T (2018) Malignant tumors associated with juvenile polyposis syndrome in Japan. Surg Today 48:253–263. https://doi.org/10.1007/s00595-017-1538-2
127. Blatter R, Tschupp B, Aretz S et al (2020) Disease expression in juvenile polyposis syndrome: a retrospective survey on a cohort of 221 European patients and comparison with a literature-derived cohort of 473 SMAD4/BMPR1A pathogenic variant carriers. Genet Med 22:1524–1532. https://doi.org/10.1038/s41436-020-0826-1
128. MacFarland SP, Ebrahimzadeh JE, Zelley K et al (2021) Phenotypic differences in juvenile polyposis syndrome with or without a disease-causing SMAD4/BMPR1A variant. Cancer Prev Res (Phila) 14:215–222. https://doi.org/10.1158/1940-6207.CAPR-20-0348
129. Howe JR, Roth S, Ringold JC et al (1998) Mutations in the SMAD4/DPC4 gene in juvenile polyposis. Science 280:1086–1088. https://doi.org/10.1126/science.280.5366.1086
130. Ma C, Giardiello FM, Montgomery EA (2014) Upper tract juvenile polyps in juvenile polyposis patients: dysplasia and malignancy are associated with foveolar, intestinal, and pyloric differentiation. Am J Surg Pathol 38:1618–1626. https://doi.org/10.1097/PAS.0000000000000283
131. Aretz S, Stienen D, Uhlhaas S et al (2007) High proportion of large genomic deletions and a genotype phenotype update in 80 unrelated families with juvenile polyposis syndrome. J Med Genet 44:702–709. https://doi.org/10.1136/jmg.2007.052506
132. Brosens LAA, van Hattem A, Hylind LM et al (2007) Risk of colorectal cancer in juvenile polyposis. Gut 56:965–967. https://doi.org/10.1136/gut.2006.116913
133. Höfting I, Pott G, Stolte M (1993) The syndrome of juvenile polyposis. Leber Magen Darm 23(107–8):111–112

134. Howe JR, Mitros FA, Summers RW (1998) The risk of gastrointestinal carcinoma in familial juvenile polyposis. Ann Surg Oncol 5:751–756. https://doi.org/10.1007/BF02303487
135. Agnifili A, Verzaro R, Gola P et al (1999) Juvenile polyposis: case report and assessment of the neoplastic risk in 271 patients reported in the literature. Dig Surg 16:161–166. https://doi.org/10.1159/000018711
136. Goff V Transparency and disclosure: the route to accountability. Issue Brief (Inst Health Care Costs Solut) 2:1–12
137. Woodford-Richens K, Bevan S, Churchman M et al (2000) Analysis of genetic and phenotypic heterogeneity in juvenile polyposis. Gut 46:656–660. https://doi.org/10.1136/gut.46.5.656
138. Achatz MI, Porter CC, Brugières L et al (2017) Cancer screening recommendations and clinical Management of Inherited Gastrointestinal Cancer Syndromes in childhood. Clin Cancer Res 23:e107–e114. https://doi.org/10.1158/1078-0432.CCR-17-0790
139. Cohen S, Hyer W, Mas E et al (2019) Management of Juvenile Polyposis Syndrome in children and adolescents: a position paper from the ESPGHAN polyposis working group. J Pediatr Gastroenterol Nutr 68:453–462. https://doi.org/10.1097/MPG.0000000000002246
140. Xu X, Jin D, Durgan J, Hall A (2013) LKB1 controls human bronchial epithelial morphogenesis through p114RhoGEF-dependent RhoA activation. Mol Cell Biol 33:2671–2682. https://doi.org/10.1128/MCB.00154-13
141. McGarrity TJ, Amos CI, Baker MJ (2001) Peutz-Jeghers Syndrome. GeneReviews® (updated 2021)
142. Lim W, Olschwang S, Keller JJ et al (2004) Relative frequency and morphology of cancers in STK11 mutation carriers. Gastroenterology 126:1788–1794. https://doi.org/10.1053/j.gastro.2004.03.014
143. Giardiello FM, Brensinger JD, Tersmette AC et al (2000) Very high risk of cancer in familial Peutz-Jeghers syndrome. Gastroenterology 119:1447–1453. https://doi.org/10.1053/gast.2000.20228
144. Pezzolesi MG, Zbuk KM, Waite KA, Eng C (2007) Comparative genomic and functional analyses reveal a novel cis-acting PTEN regulatory element as a highly conserved functional E-box motif deleted in Cowden syndrome. Hum Mol Genet 16:1058–1071. https://doi.org/10.1093/hmg/ddm053
145. Yehia L, Eng C (2001) PTEN Hamartoma Tumor Syndrome. GeneReviews® (updated 2021)
146. Mester J, Eng C (2015) Cowden syndrome: recognizing and managing a not-so-rare hereditary cancer syndrome. J Surg Oncol 111:125–130. https://doi.org/10.1002/jso.23735
147. Tan M-H, Mester JL, Ngeow J et al (2012) Lifetime cancer risks in individuals with germline PTEN mutations. Clin Cancer Res 18:400–407. https://doi.org/10.1158/1078-0432.CCR-11-2283
148. Heald B, Mester J, Rybicki L et al (2010) Frequent gastrointestinal polyps and colorectal adenocarcinomas in a prospective series of PTEN mutation carriers. Gastroenterology 139:1927–1933. https://doi.org/10.1053/j.gastro.2010.06.061
149. Ngeow J, Eng C (2015) PTEN hamartoma tumor syndrome: clinical risk assessment and management protocol. Methods 77–78:11–19. https://doi.org/10.1016/j.ymeth.2014.10.011
150. Zbuk KM, Eng C (2007) Hamartomatous polyposis syndromes. Nat Clin Pract Gastroenterol Hepatol 4:492–502. https://doi.org/10.1038/ncpgasthep0902
151. Rosty C (2018) The role of the surgical pathologist in the diagnosis of gastrointestinal polyposis syndromes. Adv Anat Pathol 25:1–13. https://doi.org/10.1097/PAP.0000000000000173
152. Adams DR, Eng CM (2018) Next-generation sequencing to diagnose suspected genetic disorders. N Engl J Med 379:1353–1362. https://doi.org/10.1056/NEJMra1711801

Computer-Assisted Interpretation of Cancer-Predisposing Variants

8

Emanuele Bonetti, Gianluca Vozza, and Luca Mazzarella

Abstract

The increasing scope of genomic profiling in cancer care has led to a specific issue related to the interpretation of genetic variants as benign or pathogenic in clinical settings for adequate patient management. In the last few years, several bioinformatic tools have been developed in order to assist the decision-making process during the evaluation of mutations in cancer-predisposing genes and the actionability of druggable variants [1–3].

8.1 The Problem of Variant Interpretation in Genetics

The increasing scope of genomic profiling in cancer care has led to a specific issue related to the interpretation of genetic variants as benign or pathogenic in clinical settings for adequate patient management. In the last few years, several bioinformatic tools have been developed in order to assist the decision-making process during the evaluation of mutations in cancer-predisposing genes and the actionability of druggable variants [1–3].

Initially, these tools were designed to assess the impact of missense mutations on the protein structure and/or function (Functional Prediction Tools, FPTs) or to evaluate the degree of conservation in the sequence in a specific site (Conservation Tools, CTs). In origin, FPTs were developed in order to evaluate the functional

E. Bonetti · G. Vozza
Department of Onco-Hematology, Università degli Studi di Milano, Milan, Italy

Department of Experimental Oncology, European Institute of Oncology IRCCS, Milan, Italy

L. Mazzarella (✉)
Department of Experimental Oncology, European Institute of Oncology IRCCS, Milan, Italy
e-mail: luca.mazzarella@ieo.it

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_8

impact of missense mutations in random mutagenesis experiments before the rise of the modern NGS technologies. For instance, the *in silico* predictor SIFT, Sorting Intolerant From Tolerant, was born in 2003 and the first human genome sequence was published in 2001 [4].

FPTs can be divided into two major categories: (1) predictive tools based on machine learning that are classification algorithms which use a set of features concerning physical and chemical properties; (2) deterministic tools that are algorithms which use scoring function and thresholds to identify amino acid substitutions and their potential impact on the protein function.

CTs, differently from FPTs, provide scores that define degree of conservation across species, with some tools focusing on mammals and other extending to vertebrates. As an example, PhyloP scores measure evolutionary conservation of a certain site in the genome considering vertebrates (phyloP100way_vertebrate, 100 vertebrates), mammals (phyloP30way_mammals, 30 mammals), and primates (phyloP17way_primate, 17 primates).

The majority of FPT algorithms implement different scoring functions and outputs that could result in a prediction like *Benign, Possibly damaging and Probably damaging* (Polyphen); *Damaging and Tolerant* (SIFT); *Disease causing, Disease causing automatic, Polymorphism and Polymorphism automatic* (Mutation Taster). A comprehensive list of FPTs and CTs is shown in Fig. 8.1 [5].

In 2011, the first comprehensive collection of functional predictions and conservation scores of putative non-synonymous Single Nucleotide Variants (nsSNVs) was published as dbNSFP. The first version contained an amount of 75,931,005 nsSNVs. In the latest version were included also splice-site SNVs reaching 84,013,490 variants [6, 7].

Alongside the development of FPTs and CTs, with the advent of NGS technologies, large-scale initiatives concurred the generation of the catalog of human genetic variation, the so-called "human variome" [8]. The first effort in this sense was the 1000 Genomes Project that resulted in the sequencing of 1092 genomes in 2012 that became 2504 in 2015. These genomes were collected worldwide in order to be representative of each ethnicity [9].

In 2016, as part of the Exome Aggregation Consortium (ExAC) published in *Nature* the first large-scale analysis of protein coding genetic variation, a collection of 60,706 human exomes made of 7,404,909 variants and their allele frequencies within the population where the majority of individuals was European [10]. Right after ExAC publication, the genome Aggregation Database (gnomAD) was released and it contained two major callsets: exome sequencing data from 123,136 individuals and whole genome sequencing from 15,496 individuals (Fig. 8.2) [11].

The assumptions of *in silico* prediction tools for which the impact of the mutation on the protein structure or the degree of conservation correlates with their intrinsic ability to be disease causing were not exact and the main reasons were: (1) the lack of known protein structures; (2) compensatory effect of other mutations. Thus, there is no general consensus in tool predictions[12]. Moreover, the introduction of population databases revealed that a huge number of variants predicted to be disease

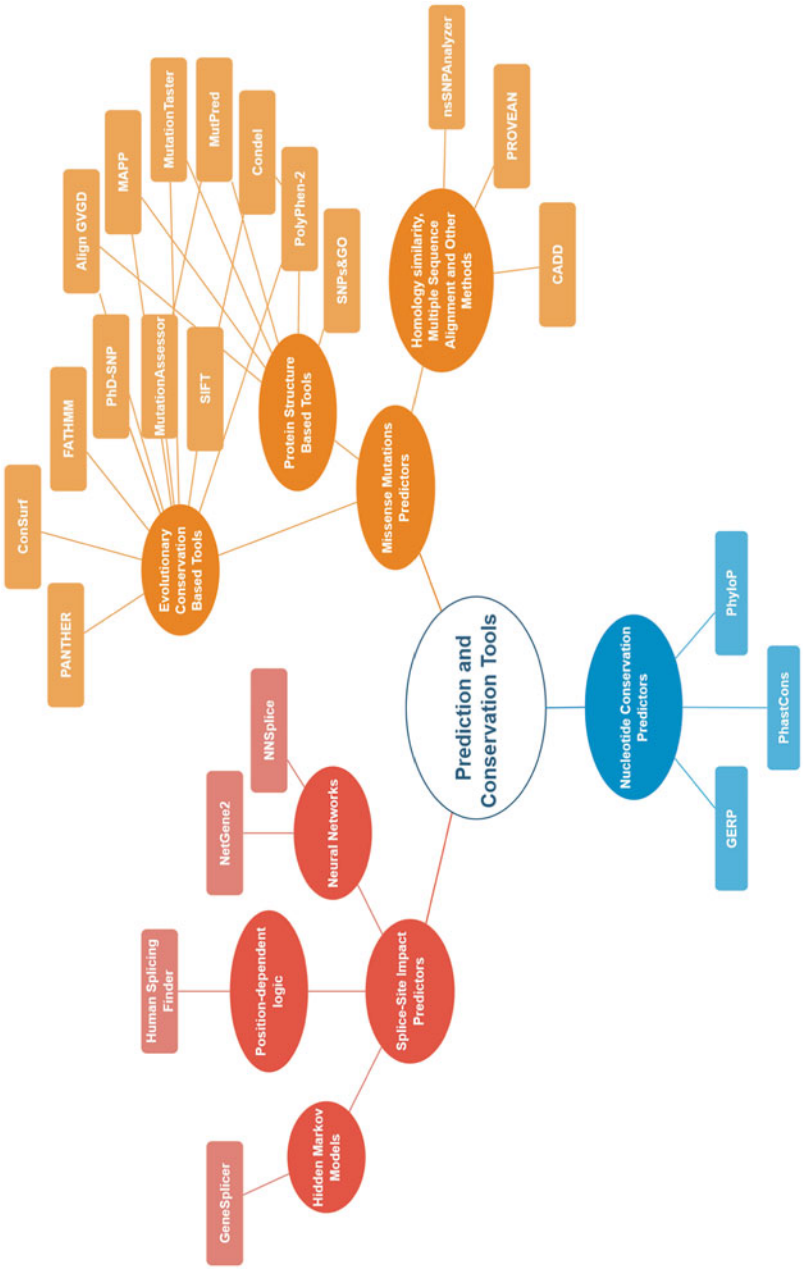


Fig. 8.1 Functional prediction and conservation tools [5]

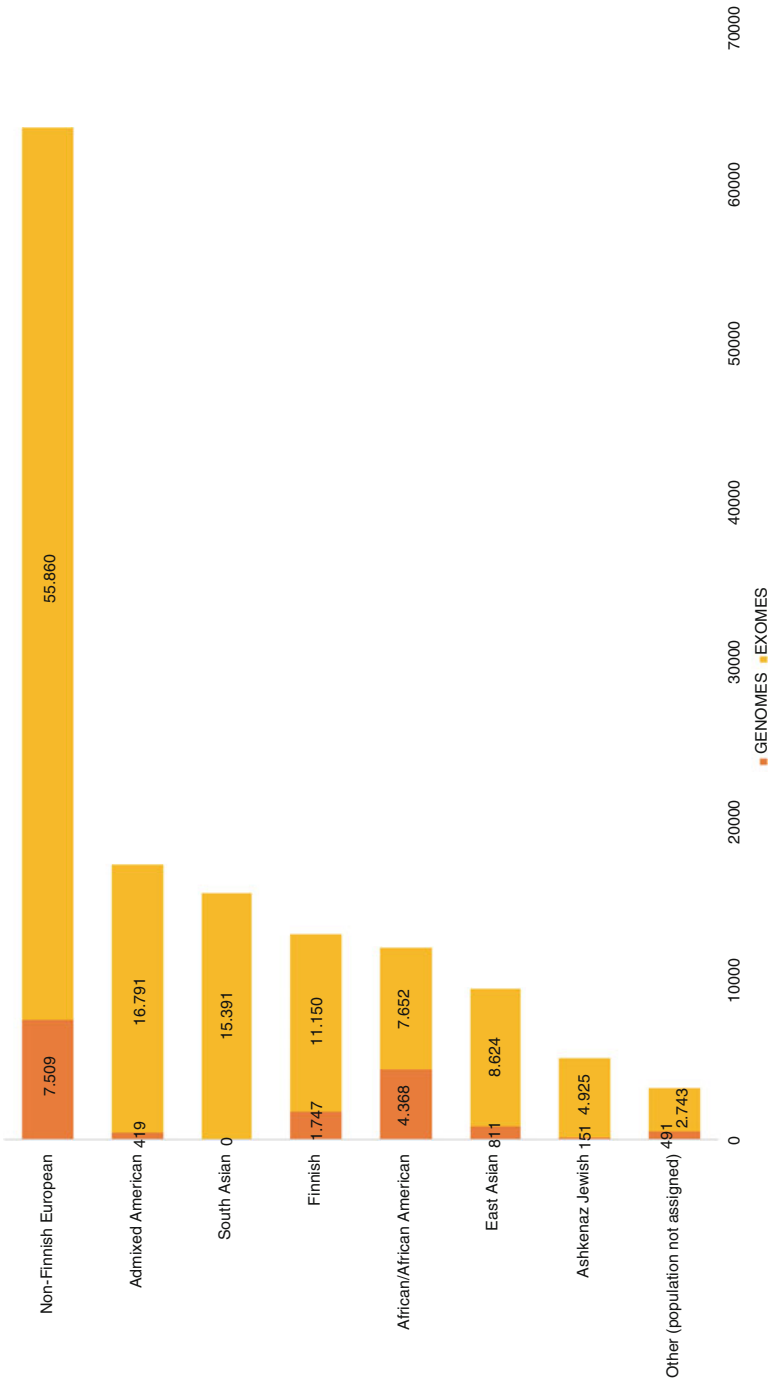


Fig. 8.2 gnomAD ethnicity distribution of exomes and genomes [11]

causing showed too high allele frequencies in sub-populations thus defining them as polymorphisms.

8.1.1 Clinical Databases

From 1996, before the development of *in silico* tools and population databases, classification of variants was based on the Human Gene Mutation Database (HGMD), the very first catalog of human variations associated with phenotypes related to human inherited diseases [13–16].

During the next two decades, several different databases were developed (Table 8.1), the most relevant one is ClinVar which is a freely accessible public archive of variants of medical interest and their phenotypes supported by evidence. So far, ClinVar is populated with 1,194,065 variants associated with 15,582 conditions (ClinVar release 2022-02-28) that are classified following the classification reported in Table 8.2 [17].

Unfortunately, the most represented significance class is "uncertain significance" (505,691) and it is easily verifiable that this number is exponentially increasing over

Table 8.1 Population, disease, and sequence databases [19]

Database	Type	Description	Website
Exome Aggregation Consortium	Population Database	Variants found in 61,486 individual exome sequencings	https://gnomad.broadinstitute.org/ It has been integrated in gnomAD
Exome Variant Server	Population Database	Variants from European and African American exomes sequencing	http://evs.gs.washington.edu/EVS
1000 Genomes	Population Database	Variants from genomic and targeted sequencing in 26 equally distributed populations. Part of the data comes from related individuals	https://www.internationalgenome.org/
dbSNP	Population Database	Short genetic variants	http://www.ncbi.nlm.nih.gov/snp
dbVar	Population Database	Structural variants	http://www.ncbi.nlm.nih.gov/dbvar
ClinVar	Disease Databases	Variants of medical interest with phenotypes and supporting evidence	http://www.ncbi.nlm.nih.gov/clinvar
OMIM	Disease Databases	Relationships between genes and genetic conditions	http://www.omim.org
NCBI Genome	Sequence Databases	Source of information on genomes (sequences, chromosomes, assemblies, and annotations)	http://www.ncbi.nlm.nih.gov/genome
RefSeqGene	Sequence Databases	Source of genomic reference standard sequences	http://www.ncbi.nlm.nih.gov/refseq/rsg

Table 8.2 ClinVar variants by category (ClinVar release 2022-02-28) [22]

Submission significance	Variants	Genes	Conditions	Submitters
Uncertain significance	505,691	17,134	7841	769
Likely benign	350,859	10,604	3702	300
Benign	239,739	15,785	5038	272
Pathogenic	137,047	11,569	9778	1566
Likely pathogenic	73,612	5428	6097	1110
Not provided	23,343	2184	1490	154
Drug response	2672	120	152	31
Other	2149	120	1127	20
Risk factor	1062	537	533	65
Association	414	187	116	45
Affects	263	99	91	17
Protective	92	65	57	9
Confers sensitivity	17	7	3	2
Association not found	2	2	1	1

time ($y = 3 \times 10^{-12} e^{(-0.0009x)}$, with $R^2 = 0.94$) [18]. Even though ClinVar has been extensively used in clinical practice, the need for an integrated classification system led to the definition of standardized guidelines for clinical variants interpretation.

8.1.2 Standard Guidelines for the Interpretation of Sequence Variations

The first guidelines for the interpretation of genetic variants were published in 2000 by the American College of Medical Genetics (ACMG), followed by a substantial update in 2007, ACMG. However, a clear turning point was marked in 2015 with the publishing of a rule-based methodology by the ACMG in a joint effort with the Association for Molecular Pathology (AMP) and the College of American Pathologists (CAP). These guidelines use standard terminology such as "benign," "likely benign," "pathogenic," "likely pathogenic," and "uncertain significance" and the process for the classification relies on criteria that use typical types of evidence for variants as population databases, disease databases (Table 8.1), *in silico* predictions tools (Fig. 8.1), functional impact information. The criteria are listed in Table 8.3 [19–21].

After the definition of a strict rule-based system for germline variant interpretation, in 2017, ACMG, AMP, American Society of Clinical Oncology (ASCO), and CAP defined a new set of rules to determine the clinical relevance in somatic variants in cancer as well. This system is based on four tiers (tier I, variant with strong clinical significance; tier II, variant with potential clinical significance; tier III, variant with uncertain clinical significance; tier IV, variant deemed to be benign).

Interpretation for germline variants is strictly focused on association between pathogenicity of a variant and disease causality. Differently, interpretation of

Table 8.3 List of ACMG-AMP-CAP 2015 guidelines criteria [19]

Criterion	Description
Pathogenic Very Strong 1 (PVS1)	Null variants in genes where loss of function is a known mechanism of disease. (non-sense, frameshift, splice-site)
Pathogenic Strong 1 (PS1)	Same amino acidic change of an established pathogenic variant regardless of nucleotide change.
Pathogenic Strong 2 (PS2)	De novo maternity/paternity confirmed variants in patients without family history.
Pathogenic Strong 3 (PS3)	Damaging effect supported by functional studies (in-vitro/in-vivo).
Pathogenic Strong 4 (PS4)	The variant prevalence in affected individuals is significantly higher compared to the controls.
Pathogenic Moderate 1 (PM1)	Variant located in pathogenic mutational hot-spot or critical functional domain
Pathogenic Moderate 2 (PM2)	Absent in population databases or at extremely low frequency if recessive.
Pathogenic Moderate 3 (PM3)	For recessive disorders, detected in trans with a pathogenic variant.
Pathogenic Moderate 4 (PM4)	Variant that causes length changes in protein as a result of in-frame deletions/insertions.
Pathogenic Moderate 5 (PM5)	Aminoacidic residue change that occurs where a missense change determined to be pathogenic has already been seen.
Pathogenic Moderate 6 (PM6)	Putative de novo without familial confirmation.
Pathogenic Supporting 1 (PP1)	Co-segregation in multiple affected family members in a previously known disease-causing gene.
Pathogenic Supporting 2 (PP2)	Missense variant in a gene in which missense variants are a common mechanism of disease.
Pathogenic Supporting 3 (PP3)	Deleterious effect on the gene product supported by *in-silico* evidence.
Pathogenic Supporting 4 (PP4)	Patient's phenotype or family history is highly specific for a disease.
Pathogenic Supporting 5 (PP5)	Variants reported as pathogenic with no available evidence to perform an independent evaluation.
Benign Stand-Alone 1 (BA1)	Allele frequency is higher than 5% in population databases.
Benign Strong 1 (BS1)	Allele frequency is greater than expected for disorder.
Benign Strong 2 (BS2)	Observed in a healthy adult individual with full penetrance expected at an early age.
Benign Strong 3 (BS3)	No damaging effect supported by functional studies (in-vitro/in-vivo).
Benign Strong 4 (BS4)	Lack of segregation in affected members of a family.
Benign Supporting 1 (BP1)	Missense variant in a gene for which primarily truncating variants are known to cause disease.
Benign Supporting 2 (BP2)	Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern.
Benign Supporting 3 (BP3)	In-frame deletions/insertions in a repetitive region without a known function.
Benign Supporting 4 (BP4)	No deleterious effect on the gene product supported by *in-silico* evidence.
Benign Supporting 5 (BP5)	Variant found in a case with an alternate molecular basis for disease.
Benign Supporting 6 (BP6)	Variants reported as benign with no available evidence to perform an independent evaluation.
Benign Supporting 7 (BP7)	A synonymous variant with no *in-silico* evidence of splicing impact.

Greeen: Benign
Red: Pathogenic

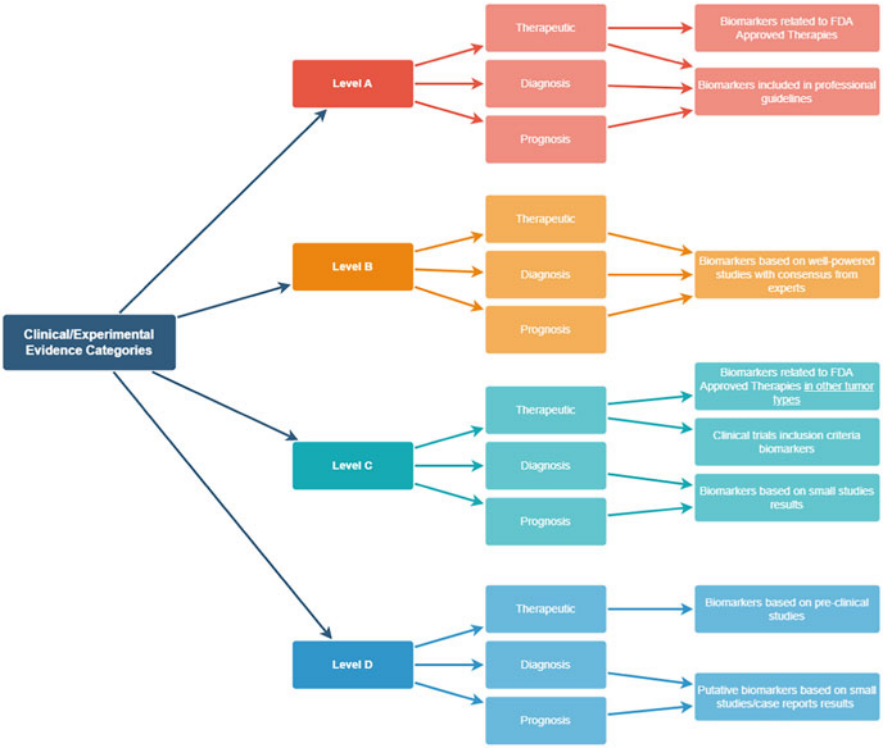


Fig. 8.3 Evidence levels in 2017 guidelines [23]

somatic variants is related to the impact of a variant on clinical care. In fact, a somatic mutation can be informative about sensitivity, resistance, and toxicity of a drug and/or a treatment; can serve as an inclusion criterion in clinical investigations; it can impact the outcome in diagnosis and prognosis assessment and in surveillance for early cancer detection. Figure 8.3 shows the levels of evidence for these criteria (levels A, B, C, D). Levels A and B are necessary to define tier I variants. Levels C and D define tier II variants [23].

8.1.3 Computer-Assisted Prioritization of Variants

The clinical relevance assessment process of genetic variations (both germline and somatic) is called prioritization [24–26].

The aforementioned criteria allowed the development of bioinformatics tools based on these standardized algorithms, namely InterVar that was developed by Quan Li and Kai Wang on the basis of 2015 guidelines for germline variants. A InterVar retrieves information about each variant from publicly available databases

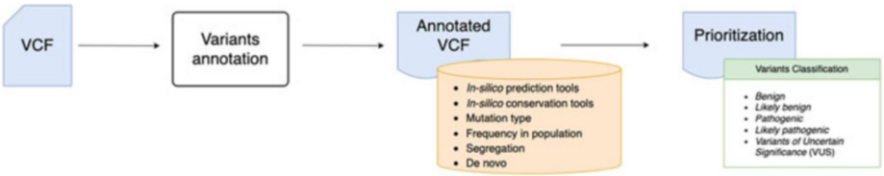


Fig. 8.4 InterVar annotation and prioritization flowchart [27]

using automatic annotation systems and it is able to compute a score based on the guidelines' criteria and then convert the score to the corresponding class [27].

The criteria provided by the standard guidelines defined in 2017 for somatic variants interpretation cannot directly be converted into a scoring system analogous to the 2015 standard guidelines for germline variants interpretation. Thus, the same InterVar authors developed CancerVar in 2020, defining a new scoring system related to the guidelines' evidence levels (A, B, C, D) that allows the somatic variants interpretation (Fig. 8.3) [28].

The consequent automation of the prioritization process is useful to reduce and even completely remove the human error bias and to reduce the time needed to assess clinical relevance to hundreds or even thousands of variants. These tools can be easily integrated in variant calling bioinformatic pipelines as well. A description of how the information is retrieved is available for InterVar in Fig. 8.4.

8.1.4 Machine Learning-Assisted Prioritization

Differently from canonical prioritization tools as InterVar that applies a standardized assessment process to define pathogenicity of variants, Machine Learning (ML)-assisted tools to relate data in a not predefined manner and generate predictions.

ML systems can be divided into two major categories: supervised and unsupervised. In this chapter, we are not going to discuss unsupervised ML. As the name suggests, supervised ML requires a so-called ground truth, in our specific case a set of variants known to be pathogenic or benign. The ground truth is divided into two sets: the training set and the test set. So, the training set is used to train the chosen algorithm to recognize the classes of interest (i.e., pathogenic and benign). The test set is a subsample of the ground truth (usually 30%) used to test the classification ability of the ML algorithm. A common caveat of ML algorithms is that they may be non-generalizable. This means that when the ML algorithm is applied outside of the ground truth, the performance could dramatically decrease (overfitting). To avoid this issue, best practice suggests testing the algorithm on external validation sets where the class is assigned in a different manner with respect to the ground truth.

In order to best know the where and when of the application of a ML algorithm, it is strongly suggested to know the parameters of the ground truth used to train and

test the algorithm itself. For instance, Learning from Evidence to Assess Pathogenicity (LEAP), a ML-based tool, starting from the ACMG guidelines integrates different features namely health conditions and history and covariates to classify the pathogenicity level of variants in a set of 24 multi-cancer predisposing genes (cit. leap paper) [29]. In this context, the training set from the ground truth used was a set of 14,226 missense variants in genes associated with elevated risk for hereditary cancer and 5398 missense variants in genes associated with elevated risk for cardiovascular disorders. The variants were classified as "pathogenic," "likely pathogenic," "benign," "likely benign," and "uncertain significance" following the 2015 ACMG guidelines and then reviewed by expert scientists and a board of certified geneticists and pathologists. It is clear that LEAP is designed to assess the pathogenicity (hence the ability of the variant to predispose to cancer or to cardiovascular diseases) only in its field of application. So, ML algorithms like LEAP should be strictly applied within their specific context of development.

By going to a broader spectrum of applications of ML, Nicora and colleagues developed a Penalized Logistic Regression-based algorithm that was trained on clinically observed genetic variants associated with a large set of diseases from several public resources (ClinVar, ARUP Mutation Database, Carver Mutation Database, and Emory Genetics Laboratory Variant Classification Catalog) [30]. The most established database in the list used in clinical practice is ClinVar. Common biases in ClinVar are the conflicting interpretations provided by multiple submitters for a single variant or the classification provided by a single submitter. In order to get rid of the classification biases in the ClinVar database, they removed all of the "conflicting interpretation of pathogenicity" (CIP) variants. The collected dataset had 5649 confirmed pathogenic variants and 8509 confirmed benign variants classified following the 2015 ACMG/AMP guidelines by submitters. Furthermore, the algorithm was trained using two approaches: (1) "A" approach that is based solely on a set of 2015 ACMG/AMP criteria ("Pathogenic Very Strong" (PVS), "Pathogenic Strong" (PS), "Pathogenic Moderate" (PM), "Pathogenic Supporting" (PP), "Benign Stand-Alone" (BA1), "Benign Strong" (BS), and "Benign Supporting" (BP)); (2) "B" approach that integrates further publicly available information about allele frequencies in population, *in silico* prediction scores.

Both approaches achieved the same results in precision and sensitivity (accuracy score "A": 97.84%, accuracy score "B": 98%) pointing out that publicly available databases are powerful resources for clinical classification of variants even when they are consequently integrated with information provided by submitters.

The algorithm that has been trained on the highest number of variants is RENOVO, a random-forest machine learning algorithm that classifies variants as pathogenic or benign based on publicly available information and provides a Pathogenicity Likelihood Score (PLS) that represents the percentage of decision trees that classified as "pathogenic" the variant of interest. PLS ranges from 0 to 1; to facilitate the interpretation, it has been converted to 6 pathogenicity intervals. The intervals are HP Benign (High Precision, 99% precision, PLS < 0.0092), IP Benign (Intermediate Precision, between 90 and 99% precision, $0.0092 \leq$ PLS < 0.235), LP Benign (Low Probability, less than 90% precision, $0.235 \leq$ PLS < 0.5), LP

Pathogenic ($0.5 \leq$ PLS < 0.7849), IP Pathogenic ($0.7849 \leq$ PLS < 0.8890), HP Pathogenic (PLS ≥ 0.8890). The training set included "stable" variants ($n = 332{,}231$) that maintained the same classification status as pathogenic/likely pathogenic or benign/likely benign over time (i.e., from the time of first insertion to the extraction date); the test set included "unstable" variants ($n = 18{,}312$), for which the status changed over time, in most cases with a conversion from CIP/VUS at the time of insertion to pathogenic/likely pathogenic or benign/likely benign at the time of extraction. Features from publicly available databases were assigned to the training set in order to cover the highest number of ACMG/AMP criteria (Table 8.4). VUS ($n = 216{,}716$) and CIP ($n = 30{,}440$) datasets have been used to test RENOVO reclassification ability on these kinds of variants that are the most difficult to handle in clinical settings. In terms of accuracy, there are no significant differences with other ML algorithms (RENOVO accuracy on training set: 98.79%; accuracy on test set: 95.28%). While other algorithms tend to slavishly follow the

Table 8.4 Table showing the ACMG criteria covered by RENOVO

Criterion	Covered in RENOVO
Pathogenic Very Strong 1 (PVS1)	YES
Pathogenic Strong 1 (PS1)	NO
Pathogenic Strong 2 (PS2)	NO
Pathogenic Strong 3 (PS3)	NO
Pathogenic Strong 4 (PS4)	NO
Pathogenic Moderate 1 (PM1)	YES
Pathogenic Moderate 2 (PM2)	YES
Pathogenic Moderate 3 (PM3)	NO
Pathogenic Moderate 4 (PM4)	YES
Pathogenic Moderate 5 (PM5)	NO
Pathogenic Moderate 6 (PM6)	NO
Pathogenic Supporting 1 (PP1)	NO
Pathogenic Supporting 2 (PP2)	YES
Pathogenic Supporting 3 (PP3)	YES
Pathogenic Supporting 4 (PP4)	NO
Pathogenic Supporting 5 (PP5)	NO
Benign Stand-Alone 1 (BA1)	YES
Benign Strong 1 (BS1)	YES
Benign Strong 2 (BS2)	YES
Benign Strong 3 (BS3)	NO
Benign Strong 4 (BS4)	NO
Benign Supporting 1 (BP1)	YES
Benign Supporting 2 (BP2)	NO
Benign Supporting 3 (BP3)	YES
Benign Supporting 4 (BP4)	YES
Benign Supporting 5 (BP5)	NO
Benign Supporting 6 (BP6)	NO
Benign Supporting 7 (BP7)	YES

Column 1 shows the criterion name and code, pathogenic criteria are red colored, benign criteria are green colored. Column 2 shows a "YES" or a "NO" if the criterion is covered or not in RENOVO [18]

2015 ACMG/AMP guidelines classification system with a slight improvement in the ability to reclassifying variants of uncertain significance (for instance, InterVar reclassify only 15% of RENOVO VUS dataset), RENOVO algorithm proposed a reclassification of 67% of ClinVar VUS/CIP with an estimated precision higher than 90% [18].

ML is a powerful resource when it comes to interpreting clinically relevant variants but it must be handled with care and the clinician must be aware that the 2015 ACMG/AMP guidelines represent the only recognized approach to variant interpretation. Publicly available data for the interpretation of variants is dramatically increasing over years. However, many of these data remain highly specific for those diseases that are most thoroughly studied due to their incidence in the population. This places some limits to the use of guidelines and even to the ML algorithms that can be biased, for instance, by unbalanced ethnicities (consider that the majority of variants reported in gnomAD come from Non-Finnish and American Europeans). On the other hand, the increasing knowledge reported in public databases could be used to retrain and keep up-to-date ML algorithms and, furthermore, apply them in a disease-specific context.

References

1. Zheng H et al (2020) Comprehensive review of web servers and bioinformatics tools for cancer prognosis analysis. Front Oncol 10. https://doi.org/10.3389/fonc.2020.00068
2. Cusin I et al (2018) A new bioinformatics tool to help assess the significance of BRCA1 variants. Hum Genomics 12(1):36. https://doi.org/10.1186/s40246-018-0168-0
3. Rogers MF, Gaunt TR, Campbell C (2021) Prediction of driver variants in the cancer genome via machine learning methodologies. Brief Bioinform 22(4). https://doi.org/10.1093/bib/bbaa250
4. Ng PC (2003) SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res 31(13):3812–3814. https://doi.org/10.1093/nar/gkg509
5. Tang H, Thomas PD (2016) Tools for predicting the functional impact of nonsynonymous genetic variation. Genetics 203(2):635–647. https://doi.org/10.1534/genetics.116.190033
6. Liu X, Jian X, Boerwinkle E (2011) dbNSFP: A lightweight database of human nonsynonymous SNPs and their functional predictions. Hum Mutat 32(8):894–899. https://doi.org/10.1002/humu.21517
7. Liu X, Li C, Mou C, Dong Y, Tu Y (2020) dbNSFP v4: a comprehensive database of transcript-specific functional predictions and annotations for human nonsynonymous and splice-site SNVs. Genome Med 12(1):103. https://doi.org/10.1186/s13073-020-00803-9
8. Chiara M, Pavesi G (2017) Evaluation of quality assessment protocols for high throughput genome resequencing data. Front Genet 8:94. https://doi.org/10.3389/fgene.2017.00094
9. Auton A et al (2015) A global reference for human genetic variation. Nature 526(7571):68–74. https://doi.org/10.1038/nature15393
10. Karczewski KJ et al (2017) The ExAC browser: displaying reference data information from over 60 000 exomes. Nucleic Acids Res. 45(D1):D840–D845. https://doi.org/10.1093/nar/gkw971
11. Karczewski KJ et al (2020) The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 581(7809):434–443. https://doi.org/10.1038/s41586-020-2308-7
12. Miosge LA et al (2015) Comparison of predicted and actual consequences of missense mutations. Proc Natl Acad Sci 112(37). https://doi.org/10.1073/pnas.1511585112

13. Stenson PD et al (2003) Human gene mutation database (HGMD ®): 2003 update. Hum Mutat 21(6):577–581. https://doi.org/10.1002/humu.10212
14. Krawczak M et al (2000) Human gene mutation database? A biomedical information and research resource. Hum Mutat 15(1):45–51. https://doi.org/10.1002/(SICI)1098-1004(200001)15:1<45::AID-HUMU10>3.0.CO;2-T
15. Cooper D (1998) The human gene mutation database. Nucleic Acids Res 26(1):285–287. https://doi.org/10.1093/nar/26.1.285
16. Krawczak M (1997) The human gene mutation database. Trends Genet 13(3):121–122. https://doi.org/10.1016/S0168-9525(97)01068-8
17. Landrum MJ et al (2014) ClinVar: public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res 42:D980–D985. https://doi.org/10.1093/nar/gkt1113
18. Favalli V et al (2021) Machine learning-based reclassification of germline variants of unknown significance: The RENOVO algorithm. Am J Hum Genet 108(4):682–695. https://doi.org/10.1016/j.ajhg.2021.03.010
19. Richards S et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17(5):405–424. https://doi.org/10.1038/gim.2015.30
20. Richards CS et al (2008) ACMG recommendations for standards for interpretation and reporting of sequence variations: revisions 2007. Genet Med 10(4):294–300. https://doi.org/10.1097/GIM.0b013e31816b5cae
21. Kazazian HH, Boehm CD, Seltzer WK (2000) ACMG recommendations for standards for interpretation of sequence variations. Genet Med 2(5):302–303. https://doi.org/10.1097/00125817-200009000-00009
22. Henrie A et al (2018) ClinVar Miner: demonstrating utility of a web-based tool for viewing and filtering ClinVar data. Hum Mutat 39(8):1051–1060. https://doi.org/10.1002/humu.23555
23. Li MM et al (2017) Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn 19(1):4–23. https://doi.org/10.1016/j.jmoldx.2016.10.002
24. Eilbeck K, Quinlan A, Yandell M (2017) Settling the score: variant prioritization and Mendelian disease. Nat Rev Genet 18(10):599–612. https://doi.org/10.1038/nrg.2017.52
25. Sefid Dashti MJ, Gamieldien J (2017) A practical guide to filtering and prioritizing genetic variants. Biotechniques 62(1):18–30. https://doi.org/10.2144/000114492
26. Roca I, Fernández-Marmiesse A, Gouveia S, Segovia M, Couce M (2018) Prioritization of variants detected by next generation sequencing according to the mutation tolerance and mutational architecture of the corresponding genes. Int J Mol Sci 19(6):1584. https://doi.org/10.3390/ijms19061584
27. Li Q, Wang K (2017) InterVar: clinical interpretation of genetic variants by the 2015 ACMG-AMP guidelines. Am J Hum Genet 100(2):267–280. https://doi.org/10.1016/j.ajhg.2017.01.004
28. Li Q, Ren Z, Cao K, Li MM, Wang K, Zhou Y (2021) CancerVar: an Artificial Intelligence empowered platform for clinical interpretation of somatic mutations in cancer. bioRxiv 2020.10.06.323162. https://doi.org/10.1101/2020.10.06.323162.
29. Lai C et al (2020) LEAP: using machine learning to support variant classification in a clinical setting. Hum Mutat 41(6):1079–1090. https://doi.org/10.1002/humu.24011
30. Nicora G, Zucca S, Limongelli I, Bellazzi R, Magni P (2022) A machine learning approach based on ACMG/AMP guidelines for genomic variant classification and prioritization. Sci Rep 12(1):2517. https://doi.org/10.1038/s41598-022-06547-3

Part III

Pathology

9 Histopathology of Hereditary Diffuse Gastric Cancer: From Grossing and 3D Microscopy to Immunophenotypic and Molecular Profiling

Irene Gullo, José Garcia-Pelaez, Rui Morais, Yuchun Ding, Carla Oliveira, and Fátima Carneiro

Abstract

Hereditary diffuse gastric cancer (HDGC) is one of the three inherited syndromes that affect primarily the stomach, besides gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) and familial intestinal gastric cancer (FIGC). HDGC is an autosomal dominant cancer syndrome, caused by germline variants in the *CDH1* and *CTNNA1* genes and is associated with an increased risk of development of diffuse gastric cancer and lobular breast cancer.

In this chapter, we focus on the pathological spectrum and multiple phenotypes of HDGC from carriers of germline *CDH1* variants. Particularly, the chapter will address the following topics: (1) histopathological features of

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/978-3-031-21317-5_9.

I. Gullo · C. Oliveira · F. Carneiro (✉)
Department of Pathology, Centro Hospitalar Universitário de São João (CHUSJ), Porto, Portugal

Department of Pathology, Faculty of Medicine of the University of Porto (FMUP), Porto, Portugal

Ipatimup – Institute of Molecular Pathology and Immunology of the University of Porto/i3S – Instituto de Investigação e Inovação em Saúde da Universidade do Porto, Porto, Portugal
e-mail: igullo@med.up.pt; carlaol@i3s.up.pt; fcarneiro@ipatimup.pt

J. Garcia-Pelaez
Ipatimup – Institute of Molecular Pathology and Immunology of the University of Porto/i3S – Instituto de Investigação e Inovação em Saúde da Universidade do Porto, Porto, Portugal
e-mail: jpelaez@ipatimup.pt

R. Morais
Department of Gastroenterology, Centro Hospitalar Universitário de São João (CHUSJ), Porto, Portugal

Y. Ding
NIHR BioResource for Translational Research, University of Cambridge, Cambridge, United Kingdom

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_9

HDGC intramucosal lesions, as detected in prophylactic total gastrectomy specimens, including the presentation of a novel three-dimensional model; (2) recommendations for handling prophylactic total gastrectomy specimens; (3) immunohistochemical and molecular characteristics of HDGC signet ring cell carcinoma *foci*; (4) macroscopic, endoscopic and histological findings of advanced HDGC; (5) morpho-molecular details distinguishing indolent and aggressive HDGC phenotypes.

9.1 Introduction

According to the recent GLOBOCAN cancer estimates, gastric cancer was responsible for over one million of new cancer cases and 769,000 deaths in 2020, being the fifth most frequently diagnosed malignancy and the fourth cause of cancer-related death in both sexes worldwide [1]. The majority of gastric cancer cases are sporadic. Familial clustering is described in up to 10% of patients and 1–3% of these cases are related to known pathogenic germline variants [2].

Gastric cancer patients present a poor prognosis when diagnosed at advanced stage. Early detection and removal by surgery or endoscopic techniques are the mainstay approaches for the effective management of gastric cancer patients. Therefore, the recognition of a possible heritable cause is of the utmost importance for identifying families with higher risk of developing gastric cancer, to ultimately provide germline variant carriers with targeted surveillance strategies and/or risk-reduction surgery.

Pathologists play an important role for the identification of heritable syndromes, by integrating information about gender, age at diagnosis, personal and family clinical history, imaging findings, tumour macroscopic appearance, histological classification, and molecular subtyping. As an example, the diagnosis of diffuse gastric cancer in a 51-year-old woman does not fulfil a recommended criterium for *CDH1*/*CTNNA1* genetic testing [3], but should prompt the search for in situ signet ring cell lesions, elsewhere in the stomach, as well as discussion with the multidisciplinary team about personal and family history of lobular breast cancer and diffuse gastric cancer. This standardized and careful approach may lead to the identification of previously unrecognized HDGC cases. On the same ground, the diagnosis of gastric cancer with lymphoid stroma and/or poorly differentiated, solid gastric cancer should raise the suspicion of the diagnosis of gastric cancer with microsatellite instability [4, 5]. In the case that cancer cells show mismatch repair protein deficiency, searching for germline mutations should be considered.

Three major autosomal dominant hereditary syndromes affect primarily the stomach: hereditary diffuse gastric cancer (HDGC), caused by *CDH1* or *CTNNA1* germline defects, gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) [6], a familial adenomatous polyposis variant with an exclusive gastric phenotype, caused by germline mutations in the promoter 1B of the *APC* gene; and

familial intestinal gastric cancer (FIGC), whose genetic cause is still poorly defined and is likely polygenic [7].

Gastric cancer is also part of the tumour spectrum of other heritable syndromes, including Lynch syndrome, familial adenomatous polyposis, juvenile polyposis, Peutz Jeghers syndrome, Li–Fraumeni syndrome and hereditary breast and ovarian cancer [8] (Table 9.1).

This chapter will be focused on the pathological spectrum and multiple phenotypes of hereditary diffuse gastric cancer from *CDH1* carriers. The clinical and pathological spectrum of other hereditable causes has been reviewed in detail elsewhere [9].

HDGC is a cancer syndrome characterized by an increased risk of diffuse gastric cancer and lobular breast cancer. The current guidelines for HDGC clinical management, published by the International Gastric Cancer Linkage Consortium (IGCLC), define HDGC by the presence of a pathogenic or likely pathogenic germline *CDH1* (which codifies E-cadherin) or *CTNNA1* (which codifies alpha-catenin) variant(s), in either an isolated individual or in a family harbouring diffuse gastric cancer. Moreover, the IGCLC acknowledged also individuals and families that are considered HDGC-like on the basis of clinical criteria but who have no identified pathogenic *CDH1* or *CTNNA1* variant(s) [3]. Similarly, hereditary lobular breast cancer (HLBC) is defined by the presence of a pathogenic *CDH1* variant in either an isolated individual with LBC or a family with one or more LBC cases in first- or second-degree relatives, but no known DGC in either situation. HLBC in *CDH1* carriers (individuals or families) is re-categorized as HDGC if signet ring cell carcinoma foci and/or its precursor lesions are identified in the stomach [3].

The HDGC genetic testing criteria have been broadened in the most recent clinical guidelines and include all individuals with a diagnosis of diffuse gastric cancer below the age of 50 or families presenting with clustering of diffuse gastric cancer and/or lobular breast cancer [3]. Moreover, all Māori with diffuse gastric cancer [21], families with diffuse gastric cancer and cleft lip/palate [22] or individuals with HDGC precursor lesions should also be tested for *CDH1* and *CTNNA1* gene mutations. For the complete set and details of the 2020 HDGC genetic testing criteria, the reader is referred to the recently updated HGDC guidelines paper [3].

9.2 The Indolent Phenotype of HDGC: 3D Modelling, Morphological, Immunohistochemical and Molecular Features

The current knowledge on HDGC initiation and development stems from the meticulous and insightful histopathological analysis of HDGC lesions found in prophylactic gastrectomy specimens. According to the model proposed by Carneiro et al in 2004, the morphological steps of development of early HDGC include: (a) in situ signet ring cell carcinoma, composed of neoplastic signet ring cells replacing the non-neoplastic gastric epithelium; (b) pagetoid spread of signet ring cells,

Table 9.1 Heritable gastric cancer syndromes, germline variants and histological subtype

Heritable GC syndrome	Lifetime risk of GC	Inheritance pattern	Causal gene(s)	Histological subtype	References
Main hereditable gastric cancer syndromes					
HDGC	33% (females) 42% (males)	AD	*CDH1* *CTNNA1*	Diffuse GC	[10, 11]
GAPPS	13%	AD	*APC* Promoter 1B	Intestinal and mixed GC arising in the context of Fundic gland polyposis of the proximal stomach	[6]
FIGC	66%	AD	Probable polygenic cause	Intestinal GC	[7]
Hereditable syndromes also associated with increased risk of gastric cancer					
FAP	4–7% (Asian population) Not increased in Western countries	AD	*APC*	Intestinal GC arising from intestinal-type and pyloric gland adenomas	[12, 13]
Lynch syndrome	Up to 10% (more frequent in *MLH1* and *MSH2* carriers)	AD	*MLH1* *MSH2* *MSH6* *PMS2* *EPCAM*	Intestinal GC (most cases)	[14, 15]
Peutz-Jeghers syndrome	29%	AD	*STK11*	Intestinal GC	[16]
Juvenile polyposis	10–30%	AD	*SMAD4* *BMPR1A*	Intestinal or diffuse GC arising from juvenile polyps with dysplasia	[17]
Li-Fraumeni syndrome	2–5%	AD	*TP53*	Intestinal or diffuse GC	[18]
Hereditary breast and ovarian cancer syndrome	2%	AD	*BRCA1* *BRCA2*	Intestinal GC	[19]
MAP	2% (females) 5% (males)	AR	*MUTYH*	Intestinal GC	[20]

AD, autosomal dominant; *FAP*, familial adenomatous polyposis; *FGPs*, fundic gland polyps; *FIGC*, familial intestinal gastric cancer; *GAPPS*, gastric adenocarcinoma and proximal polyposis of the stomach; *GC*, gastric cancer; *HDGC*, hereditary diffuse gastric cancer

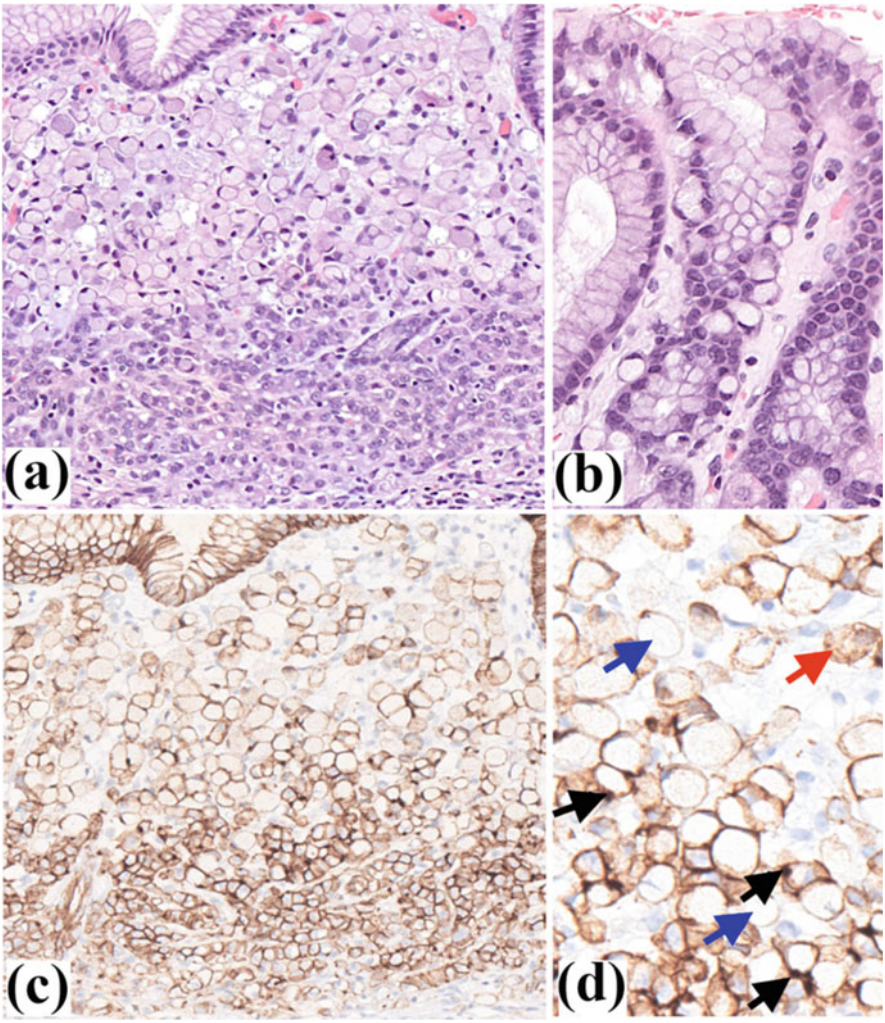


Fig. 9.1 Precursor and indolent lesions in HDGC: (**a**) intramucosal signet ring cell carcinoma focus with layered structure; (**b**) pagetoid progression of signet ring cells; (**c**, **d**) E-cadherin immunoreactivity in intramucosal signet ring cell carcinoma; although expression seems to be preserved at low magnification (**c**), a closer inspection (**d**) revealed abnormal E-cadherin expression in signet ring cells, including cytoplasmic expression (red arrow), dotted membranous expression (black arrows) and faint/absent membranous expression (blue arrows)

constituted by a row of signet ring cells located between the preserved gastric epithelium and the basal membrane; (c) early intramucosal invasive signet ring cell carcinoma, in which signet ring cells invade the lamina propria [23] (Fig. 9.1).

In situ signet ring cell carcinoma and pagetoid spread of signet ring cells are pre-invasive (in situ) lesions that, by definition, are contained within the basement

membrane of gastric epithelium. The detection rate of in situ signet ring cell carcinoma and pagetoid spread of signet ring cells is about 30%, not as frequent as the detection of intramucosal cancer *foci* (95.3%), suggesting that invasion of the lamina propria may occur without morphologically detectable pre-invasive lesion (s) [23, 24]. For the histopathological diagnosis of these rare lesions, it is advised to seek for a second opinion by an independent pathologist with experience in the field [3], in order to reduce the risk of false-positive diagnoses of nonspecific changes that may mimic signet ring cell lesions.

Intramucosal signet ring cell carcinoma *foci* disrupt the basal membrane to invade the lamina propria. Tiny *foci* usually invade the superficial portion of the gastric mucosa, while larger *foci* may involve the whole thickness of the mucosa and usually display a layered structure, with large signet ring cells with abundant mucin at the luminal surface, that progressively decrease the content of the cytoplasmic vacuoles towards the base of the lesion (Fig. 9.1). The comparison of the chromosomal aberrations of superficial and deep neoplastic cells in HDGC lesions failed to demonstrate distinct molecular profiles between the two cell types [25], suggesting that both represent different phenotypes within the spectrum of "differentiated" signet ring cell carcinoma [9].

With the help of computational science, we were able to generate a three-dimensional model of signet ring cell carcinoma *foci* within one gastric section from a *CDH1* c.1901C > T variant carrier (unpublished data). The results from the 3D reconstruction support the concept of the tumour multifocality of HDGC and the heterogeneity of the distribution of the lesions, as shown in one single section (Fig. 9.2, Supplementary Videos 9.1 and 9.2).

Consistent with the E-cadherin loss of function due to the bi-allelic inactivation of *CDH1*, E-cadherin expression is abnormal in most of precursor lesions in *CDH1* carriers [23, 26, 27]. However, intramucosal carcinoma *foci* may harbour a heterogenous pattern of E-cadherin expression, which may vary from completely absent to membranous and preserved expression. Abnormal E-cadherin staining patterns in intramucosal lesions include cytoplasmic, incomplete, dotted and/or attenuated membranous staining and perinuclear immunoreactivity (Fig. 9.1). Taking into account the heterogeneity of E-cadherin expression in intramucosal lesions, pathologists should not use E-cadherin immunohistochemical expression as a surrogate biomarker for the differential diagnosis of diffuse gastric cancer in the sporadic versus hereditary context.

Intramucosal signet ring cell *foci* in *CDH1* carriers are considered indolent lesions with low potential of progression into advanced cancer. Bearing in mind the high rate of *CDH1* carriers not developing advanced lesions (170/174, 97.7%) [24], intramucosal signet ring cell carcinoma *foci* most probably have a long latency period before (even if) progressing to advanced diffuse gastric cancer. Moreover, several immunohistochemical studies showed that intramucosal lesions show low proliferation [28–30] and express biomarkers of terminally differentiated cells [29, 31, 32]. From the molecular pathology standpoint, Nasri et al. found that intramucosal signet ring cell carcinoma *foci* share molecular features of precancerous lesions, including low levels of genomic instability and small chromosomal

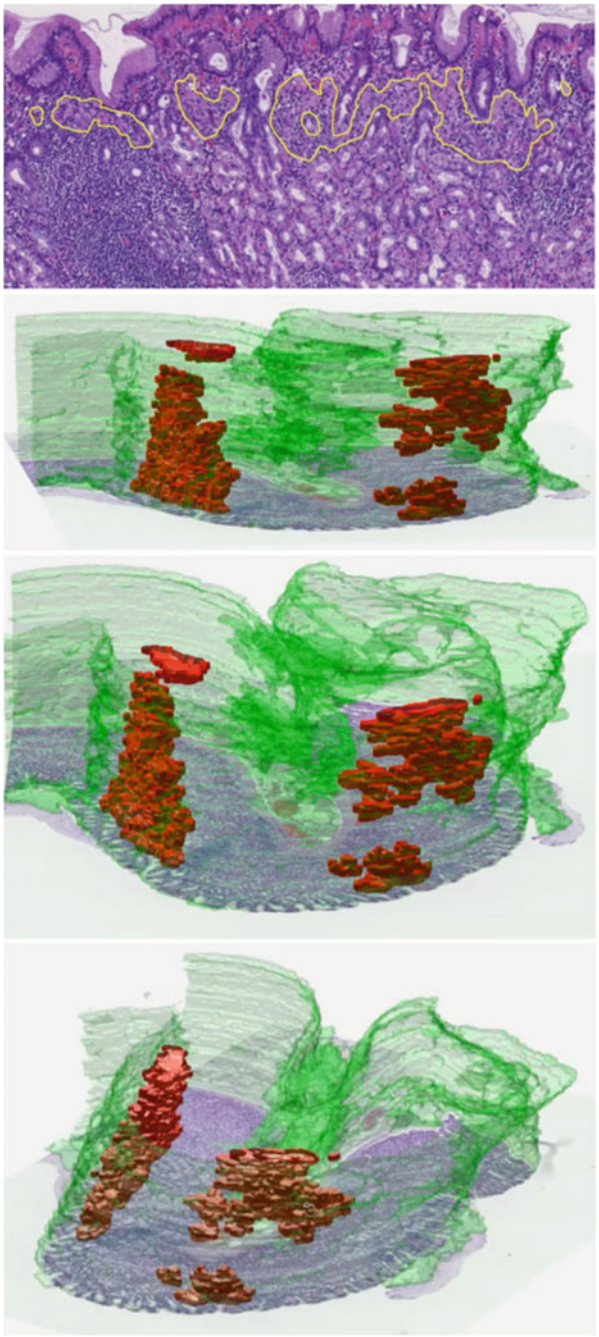


Fig. 9.2 Three-dimensional reconstruction of HDGC intramucosal foci. After cutting of several sections (~300) and selection of intramucosal signet ring cell carcinoma foci in haematoxylin and eosin-stained slides (upper figure), a 3D reconstruction was created. The green area represents the boundaries of normal gastric mucosa, while red areas represent invasive intramucosal cancer foci. Foci were multiple and at different levels within the same fragment

aberrations enriched at fragile sites, rather than molecular characteristics of overt malignancy (aneuploidy or large-scale chromosomal rearrangements) [25].

In a recent study by David Huntsman and collaborators [33], single-cell transcriptomic analysis was performed in a murine organoid model of HDGC. *CDH1* loss resulted in alterations in genes involved in cell junction organization and extracellular matrix production, including upregulation of *Krt7* and *Krt19*. Further, in prophylactic gastrectomy specimens from known carriers of *CDH1* pathogenic germline variants, intramucosal signet ring cell carcinoma *foci* showed aberrant expression of E-cadherin as well as concomitant overexpression of CK7. Although the number of lesions from *CDH1* carriers analysed in this study was not disclosed, CK7 immunohistochemistry may represent a promising biomarker for detection of signet ring cells in the gastric mucosa of *CDH1* carriers (Fig. 9.3).

9.3 Prophylactic Total Gastrectomy in *CDH1* Carriers

Carriers of *CDH1* variants from families with confirmed HDGC are advised to undergo prophylactic total gastrectomy regardless of endoscopic findings, since currently there are no proven effective gastric cancer screening strategies for *CDH1* carriers [3]. Prophylactic total gastrectomy can also be considered in *CDH1* carriers from families with hereditary lobular breast cancer (HLBC) phenotype and in *CTNNA1* carriers from HDGC families. Some patients may wish or need to postpone surgery and undergo endoscopic surveillance. In this case, patients and clinicians should consider that endoscopic evaluation of gastric mucosa is challenging in HDGC patients, because lesions spread beneath the intact foveolar epithelium and within non-neoplastic glands: several studies have shown that the yield of detection of early HDGC lesions by endoscopy is variable and below 50%, even in centres with experienced endoscopists, pathologists and high-definition endoscopy [34]. This *rationale* is behind the current clinical guidelines for HDGC management, published by the International Gastric Cancer Linkage Consortium (IGCLC), recommending prophylactic total gastrectomy as the only curative approach in HDGC patients [3].

Single or multiple signet ring cell carcinoma *foci* are detected in the large majority (~95%) of prophylactic total gastrectomies from *CDH1* carriers [24]. Visible lesions are found in a minority of cases (12%) and may appear on macroscopic examination as pale patches, nodules and tiny ulcers or scars [24]. The detection of lesions not visible to the naked eye, as well as the meticulous description of cancer *foci* number, dimension and location are dependent upon the application of rigorous grossing and sampling protocols.

The gold standard practice, according to the IGCLC, includes sectioning and embedding of the entirety of the total gastrectomy specimen, and recording the location of each tissue block (using a photo template, a schematic chart, etc.). Histopathological examination of the entire gastric mucosa, preferably performed by a pathologist with experience in the field, is also considered the gold standard practice. A recent study, reviewing data from 174 prophylactic total gastrectomy

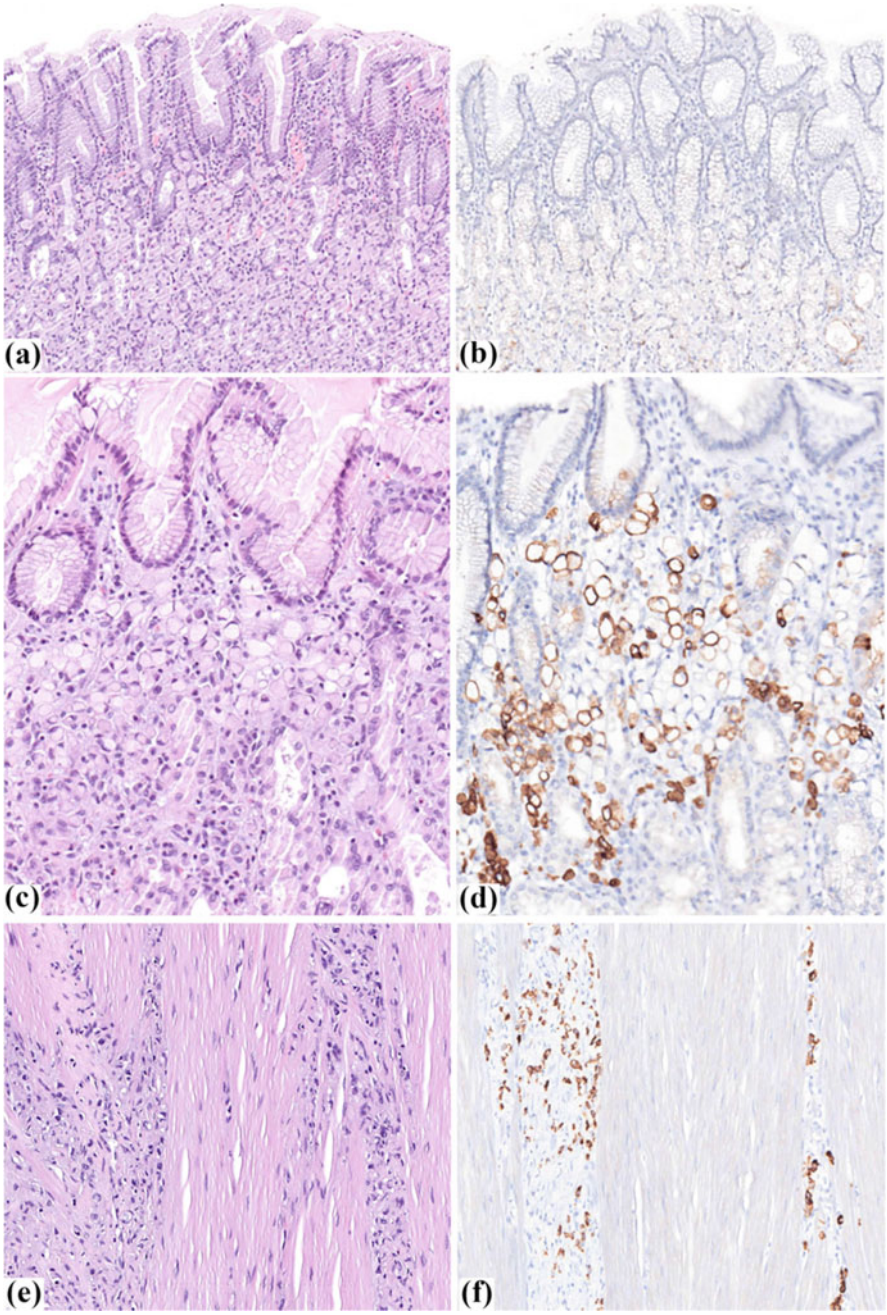


Fig. 9.3 CK7 expression in non-neoplastic mucosa and diffuse gastric cancer in a CDH1 carrier: (**a**, **b**) normal gastric mucosa does not show CK7 expression, while (**c**, **d**) intramucosal and (**e**, **f**) advanced HDGC lesions display CK7 expression

specimens in *CDH1* carriers, found that when the entire gastric mucosa is not examined, histopathological analysis may fail to detect lesions in up to 37% of cases. In contrast, when the entirety of the gastric mucosa is examined, only 5% of specimens are classified as negative [24].

The IGCLC acknowledged that for routine histopathology laboratories with constrained resources, total examination of the gastric mucosa may be not feasible. Therefore, it has been established that the pathological diagnosis should include the following minimal elements: (1) lymph nodes *status*, (2) sections of gastric mucosa from all gastric regions and (3) surgical margins, to confirm that the totality of gastric mucosa has been resected. If the gastric mucosa is not analysed in its entirety and no precursor or invasive lesions are identified, the report should not define the specimen as "negative". The pathologist should estimate the percentage of the gastric mucosa analysed and state that no precursor lesions or invasive carcinoma were identified after the analysis of several sections (specifying the number of sections collected for analysis).

The preferred approach for the analysis of prophylactic total gastrectomies, as proposed by the IGCLC, represents a compromise between diagnostic practice and research. According to this approach, the gastric mucosa is embedded in its entirety, but the pathologist may stop examining the gastric mucosa when at least one invasive carcinoma *focus* is detected. The remaining tissue blocks of the totality of the gastric mucosa are kept in the tissue archive for further research [3].

The anatomical localization of the cancer *foci* is variable and discordant results have been published: several authors reported a proximal clustering [30, 35–38], while others showed no restriction to any specific topographic region of the stomach [39, 40]. Curiously, in New Zealand Māori kindred most of the *foci* were found within the body-antrum transitional zone and distal stomach [41, 42]. The number and dimension of cancer *foci* are also variable both within and between kindreds, and are not related to age or gender of *CDH1* carriers, ranging from one to hundreds of tiny (<0.1 mm to 16 mm) *foci* [23, 24]. The cause of this variability remains to be clarified.

Prophylactic total gastrectomy specimens may harbour gastric carcinoma invading beyond the lamina propria in approximately 1–5% of cases. In a series encompassing 174 specimens, four cases showed invasion beyond the mucosa and, in one case, with invasion of the subserosa (pT3), lymph node metastases were identified. Of these, endoscopy failed to detect the invasive lesion in two cases (40%) [24].

Artificial intelligence applied to digital and computational pathology may represent a time-saving, innovative methodology to screen haematoxylin and eosin sections from prophylactic total gastrectomy specimens and assist pathologists to detect HDGC lesions. Several authors have developed deep learning models for the detection of signet ring cell with high sensitivity and very low false positive rate, even in the context of hereditary diffuse gastric cancer [43–46]. The application of artificial intelligence on *CDH1* gastrectomy specimens might represent the future solution for a rapid and cost-effective screening.

9.4 HDGC Progression: Aggressive Gastric Phenotype in *CDH1* Carriers

The picture of HDGC diagnosed at advanced, symptomatic stage (usually in family probands) is completely different from intramucosal lesions. Along progression and invasion of the gastric wall, the neoplastic cells acquire an aggressive/poorly differentiated phenotype and may rapidly spread into the peritoneal cavity and lymph nodes.

The stomach wall is diffusely infiltrated by cancer cells and appears thickened and rigid (so-called "leather bottle" stomach or *linitis plastica*). Despite the deep invasion of the gastric wall, the surface foveolar epithelium and superficial lamina propria may be intact, also in advanced lesions, and *linitis plastica* may be underdiagnosed during endoscopic examination, as well as superficial endoscopic biopsies may fail to detect cancer cells [47]. Endoscopic ultrasonography combined with fine needle aspiration biopsies of the gastric wall and/or suspicious lymph nodes may represent useful diagnostic tools to detect such deeper lesions [48–50]. Endoscopy features that may rise the suspicion of *linitis plastica* include poor distensibility and difficulty of insufflation, large and thickened gastric folds, multiple erosions and ulcers, stenosis and diffuse infiltration of the stomach, as well as thickening of the gastric wall with layer fusion by endoscopic ultrasonography [51, 52] (Fig. 9.4).

Besides displaying "aggressive" histopathological features (Fig. 9.4), advanced lesions show high proliferative activity and biomarkers of different oncogenic events, including p53 aberrant expression, c-Src kinase activation and p16 overexpression [28, 29, 31, 53]. These data suggest that aggressive features in HDGC may be triggered by complex molecular aberrations. Therefore, large chromosomal rearrangements, as well as epigenetic events, not studied in detail so far, may represent a relevant mechanism to explain the transition from indolent to

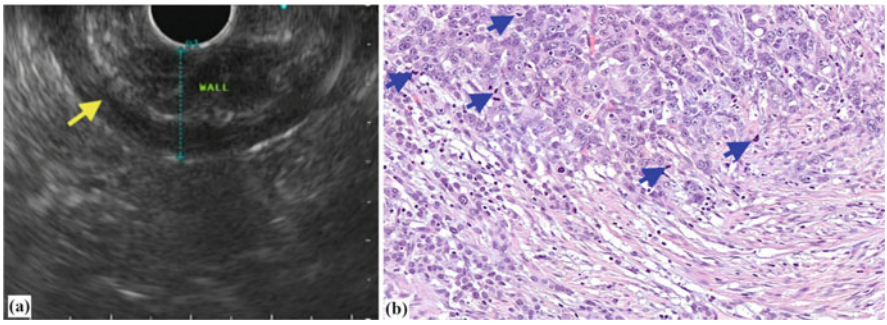


Fig. 9.4 Advanced HDGC infiltrating the gastric wall: (**a**) endoscopic ultrasonography revealing diffuse thickening of the gastric wall (13.5 mm), particularly due to expansion of the mucosa and muscularis propria, and partial layer fusion (arrow); (**b**) the tumour displays poorly differentiated, pleomorphic poorly cohesive cancer cells with high mitotic activity (blue arrows) infiltrating the muscularis propria

aggressive behaviour [54]. Particularly, concomitant molecular aberrations of *CDH1* and *TP53* could cooperate to the development of HDGC aggressive phenotypes and may be the central players for the cascade of molecular events important for HDGC progression, as observed in murine models [55] and in organoids derived from sporadic gastric cancers [56].

In the study of gastrectomy specimens, the pathologists have the important role of examining thoroughly the gastric mucosa distant from the tumour bulk: the finding of multifocal intramucosal *foci* and precursor lesions are important clues for the presence of *CDH1* germline variants [57] and are now part of the criteria for *CDH1/CTNNA1* genetic testing [3].

9.5 Conclusion

Pathologists have a fundamental role in recognizing hereditary cancer syndromes. In the setting of HDGC, the detection of in situ signet ring cell lesions and multiple signet ring cell carcinoma *foci*, especially distant from the tumour bulk, should raise the suspicion of HDGC and lead to appropriate genetic testing.

The role of pathologists, both in the clinical and research settings, is also to recognize distinct phenotypes of diseases. As described in this chapter, HDGC is a very heterogenous disease showing a plethora of morphological and molecular phenotypes with prognostic and therapeutic relevance. As an example, the finding of "aggressive" morphological and immunophenotypic/molecular features in endoscopic biopsies from *CDH1* carriers is suggestive of advanced disease and should be reported by the pathologist to prompt staging and clinical intervention.

A comprehensive analysis of the molecular landscape of indolent and aggressive lesions from *CDH1* carriers, in the frame of detailed morphologic analysis by a pathologist with experience in this field, should be the cornerstone of future studies for the discovery of new biomarkers for HDGC early detection and progression.

Acknowledgements None.

Conflicts of Interest The authors state that they have no potential conflicts of interest.

References

1. Ferlay J, Ervik M, Lam F et al (2020) Global cancer observatory: cancer today. Lyon, France: International Agency for Research on Cancer. https://gco.iarc.fr/today. Accessed 03 July 2022
2. Oliveira C, Pinheiro H, Figueiredo J et al (2015) Familial gastric cancer: genetic susceptibility, pathology, and implications for management. Lancet Oncol 16:e60–e70
3. Blair VR, McLeod M, Carneiro F et al (2020) Hereditary diffuse gastric cancer: updated clinical practice guidelines. Lancet Oncol 21:e386
4. Gullo I, Oliveira P, Athelogou M et al (2019) New insights into the inflamed tumor immune microenvironment of gastric cancer with lymphoid stroma: from morphology and digital analysis to gene expression. Gastric Cancer 22:77–90
5. Tomio A, Urara S, Motoji S et al (2013) Frequent microsatellite instability in papillary and solid-type, poorly differentiated adenocarcinomas of the stomach. Gastric Cancer 16:505

6. Worthley DL, Phillips KD, Wayte N et al (2012) Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS): a new autosomal dominant syndrome. Gut 61:774–779
7. Carvalho J, Oliveira P, Senz J et al (2020) Redefinition of familial intestinal gastric cancer: clinical and genetic perspectives. J Med Genet 58:1
8. W.H.O. classification of tumours (2019) Digestive System Tumours. Genetic tumour syndromes of the digestive system, 5th edn. WHO Classification of Tumours Editorial Board, pp 511–550
9. Gullo I, van der Post R, Carneiro F (2021) Recent advances in the pathology of heritable gastric cancer syndromes. Histopathology 78:125
10. Roberts ME, Ranola JMO, Marshall ML et al (2019) Comparison of CDH1 penetrance estimates in clinically ascertained families vs families ascertained for multiple gastric cancers. JAMA Oncol 5:1325–1331
11. van der Post RS, Vogelaar IP, Carneiro F et al (2015) Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. J Med Genet 52:361
12. Park JG, Park KJ, Ahn YO et al (1992) Risk of gastric cancer among Korean familial adenomatous polyposis patients. Report of three cases. Dis Colon Rectum 35:996–998
13. Shibata C, Ogawa H, Miura K et al (2013) Clinical characteristics of gastric cancer in patients with familial adenomatous polyposis. Tohoku J Exp Med 229:143–146
14. Moller P, Seppala TT, Bernstein I et al (2018) Cancer risk and survival in path_MMR carriers by gene and gender up to 75 years of age: a report from the prospective lynch syndrome database. Gut 67:1306–1316
15. Kim J, Braun D, Ukaegbu C et al (2020) Clinical factors associated with gastric cancer in individuals with lynch syndrome. Clin Gastroenterol Hepatol 18:830–7.e1
16. Boland CR, Idos GE, Durno C et al (2022) Diagnosis and management of cancer risk in the gastrointestinal hamartomatous polyposis syndromes: recommendations from the US multi-society task force on colorectal cancer. Gastroenterology 162:2063
17. Syngal S, Brand RE, Church JM et al (2015) ACG clinical guideline: genetic testing and management of hereditary gastrointestinal cancer syndromes. Am J Gastroenterol 110:223–62; quiz 63
18. Masciari S, Dewanwala A, Stoffel EM et al (2011) Gastric cancer in individuals with Li-Fraumeni syndrome. Genet Med 13:651–657
19. Friedenson B (2005) BRCA1 and BRCA2 pathways and the risk of cancers other than breast or ovarian. MedGenMed 7:60
20. Win AK, Reece JC, Dowty JG et al (2016) Risk of extracolonic cancers for people with biallelic and monoallelic mutations in MUTYH. Int J Cancer 139:1557–1563
21. Hakkaart C, Ellison-Loschmann L, Day R et al (2019) Germline CDH1 mutations are a significant contributor to the high frequency of early-onset diffuse gastric cancer cases in New Zealand Māori. Familial Cancer 18:83–90
22. Frebourg T, Oliveira C, Hochain P et al (2006) Cleft lip/palate and CDH1/E-cadherin mutations in families with hereditary diffuse gastric cancer. J Med Genet 43:138–142
23. Carneiro F, Huntsman DG, Smyrk TC et al (2004) Model of the early development of diffuse gastric cancer in E-cadherin mutation carriers and its implications for patient screening. J Pathol 203:681–687
24. Rocha JP, Gullo I, Wen X et al (2018) Pathological features of total gastrectomy specimens from asymptomatic hereditary diffuse gastric cancer patients and implications for clinical management. Histopathology 73:878–886
25. Nasri S, Humara B, Anjomshoaa A et al (2019) Early hereditary diffuse gastric cancer (eHDGC) is characterized by subtle genomic instability and active DNA damage response. Pathol Oncol Res 25:711–721
26. Bruner HC, Derksen PWB (2018) Loss of E-cadherin-dependent cell-cell adhesion and the development and progression of cancer, vol 10. Cold Spring Harb Perspect Biol

27. Tsugeno Y, Nakano K, Nakajima T et al (2020) Histopathologic analysis of signet-ring cell carcinoma in situ in patients with hereditary diffuse gastric cancer. Am J Surg Pathol 44:1204–1212
28. van der Post RS, Gullo I, Oliveira C et al (2016) Histopathological, molecular, and genetic profile of hereditary diffuse gastric cancer: current knowledge and challenges for the future. Adv Exp Med Biol 908:371–391
29. Humar B, Fukuzawa R, Blair V et al (2007) Destabilized adhesion in the gastric proliferative zone and c-Src kinase activation mark the development of early diffuse gastric cancer. Cancer Res 67:2480–2489
30. Barber ME, Save V, Carneiro F et al (2008) Histopathological and molecular analysis of gastrectomy specimens from hereditary diffuse gastric cancer patients has implications for endoscopic surveillance of individuals at risk. J Pathol 216:286–294
31. Lee HE, Smyrk TC, Zhang L (2018) Histologic and immunohistochemical differences between hereditary and sporadic diffuse gastric carcinoma. Hum Pathol 74:64–72
32. Gullo I, van der Post RS, Carneiro F (2020) Recent advances in the pathology of heritable cancer syndromes. Histopathology (In Press)
33. Dixon K, Brew T, Farnell D et al (2021) Modelling hereditary diffuse gastric cancer initiation using transgenic mouse-derived gastric organoids and single-cell sequencing. J Pathol 254:254
34. Gullo I, Oliveira C, van der Post RS et al (2021) Updated perspective and directions on hereditary diffuse gastric cancer. In: Jenkins B (ed) Research and clinical applications of targeting gastric neoplasms. Academic Press, pp 217–258
35. Lim YC, di Pietro M, O'Donovan M et al (2014) Prospective cohort study assessing outcomes of patients from families fulfilling criteria for hereditary diffuse gastric cancer undergoing endoscopic surveillance. Gastrointest Endosc 80:78–87
36. Rogers WM, Dobo E, Norton JA et al (2008) Risk-reducing total gastrectomy for germline mutations in E-cadherin (CDH1): pathologic findings with clinical implications. Am J Surg Pathol 32:799–809
37. Fujita H, Lennerz JK, Chung DC et al (2012) Endoscopic surveillance of patients with hereditary diffuse gastric cancer: biopsy recommendations after topographic distribution of cancer foci in a series of 10 CDH1-mutated gastrectomies. Am J Surg Pathol 36:1709–1717
38. Zhang Q, Yang Z, Karamchandani DM (2020) Complete histopathologic examination of risk reduction gastrectomy specimens for CDH1 germline mutation: is it warranted in routine clinical practice? Ann Diagn Pathol 45:151473
39. Funakoshi T, Miyamoto S, Kakiuchi N et al (2019) Genetic analysis of a case of Helicobacter pylori-uninfected intramucosal gastric cancer in a family with hereditary diffuse gastric cancer. Gastric Cancer 22:892–898
40. Huntsman DG, Carneiro F, Lewis FR et al (2001) Early gastric cancer in young, asymptomatic carriers of germ-line E-cadherin mutations. N Engl J Med 344:1904–1909
41. Charlton A, Blair V, Shaw D et al (2004) Hereditary diffuse gastric cancer: predominance of multiple foci of signet ring cell carcinoma in distal stomach and transitional zone. Gut 53:814–820
42. Blair V, Martin I, Shaw D et al (2006) Hereditary diffuse gastric cancer: diagnosis and management. Clin Gastroenterol Hepatol 4:262–275
43. Rasmussen S, Arnason T, Huang W (2021) Deep learning for computer-assisted diagnosis of hereditary diffuse gastric cancer. J Pathol Transl Med 55
44. Zhang S, Yuan Z, Wang Y et al (2021) REUR: a unified deep framework for signet ring cell detection in low-resolution pathological images. Comput Biol Med 136:104711
45. Chen Z, Wang S, Jia C et al (2021) CRDet: improving signet ring cell detection by reinforcing the classification branch. J Comput Biol 28:732
46. Kanavati F, Ichihara S, Rambeau M et al (2021) Deep learning models for gastric signet ring cell carcinoma classification in whole slide images. Technol Cancer Res Treat 20: 153303382110279

47. Shan G, Xu G, Li Y (2013) Endoscopic ultrasonographic features of gastric linitis plastica in fifty-five Chinese patients. J Zhejiang Univ Sci B 14
48. Carter J, Nelson J, Eves M et al (2008) Diagnosis of linitis plastica-type gastric adenocarcinoma by endoscopic ultrasound-guided fine needle aspiration: a case report. Acta Cytol 52:725
49. Liu Y, Chen K, Yang X (2019) Endoscopic ultrasound-guided fine-needle aspiration used in diagnosing gastric linitis plastica: metastatic lymph nodes can be valuable targets. J Gastroenterol Hepatol 34
50. Okamoto T, Suzuki H, Fukuda K (2021) Gastric linitis plastica due to signet-ring cell carcinoma with Krukenberg tumors diagnosed by endoscopic ultrasound-guided fine-needle aspiration. Clin J Gastroenterol 14
51. Gd S, Gq X, Ym L (2013) Endoscopic ultrasonographic features of gastric linitis plastica in fifty-five Chinese patients. J Zhejiang Univ Sci B 14
52. Vivier-Chicoteau J, Lambert J, Coriat R et al (2020) Development and internal validation of a diagnostic score for gastric linitis plastica. Gastric Cancer 23:639
53. Gullo I, Devezas V, Baptista M et al (2018) Phenotypic heterogeneity of hereditary diffuse gastric cancer: report of a family with early-onset disease. Gastrointest Endosc 87:1566–1575
54. Oliveira C, Sousa S, Pinheiro H et al (2009) Quantification of epigenetic and genetic 2nd hits in CDH1 during hereditary diffuse gastric cancer syndrome progression. Gastroenterology 136: 2137–2148
55. Shimada S, Mimata A, Sekine M et al (2012) Synergistic tumour suppressor activity of E-cadherin and p53 in a conditional mouse model for metastatic diffuse-type gastric cancer. Gut 61:344–353
56. Nanki K, Toshimitsu K, Takano A et al (2018) Divergent routes toward Wnt and R-spondin Niche independency during human gastric carcinogenesis. Cell 174:856–69.e17
57. Oliveira C, Moreira H, Seruca R et al (2005) Role of pathology in the identification of hereditary diffuse gastric cancer: report of a Portuguese family. Virchows Archiv 446:181

HER2 Testing in Breast and Gastric Cancer with *CDH1* Germline Mutations

10

Mariia Ivanova, Elham Sajjadi, Lorenzo Zattoni, and Nicola Fusco

Abstract

The role of human epidermal growth factor receptor-2 (HER2) is involved in epithelial cells growth and differentiation and has been widely studied in a plethora of cancers. HER2 alterations have been known to drive tumor cell proliferation, migration and reduce apoptosis. Tumor expression of HER2 marker has prognostic and predictive significance in breast and gastroesophageal cancers. These two cancer types may arise as constituents of hereditary diffuse gastric cancer syndrome (HDGS), which is linked to E-cadherin coding *CDH1* gene alterations. Precisely, HDGS features a high prevalence of diffuse gastric and lobular breast cancers. HER2 alterations may simultaneously occur with *CDH1* mutations; however, the role of HER2 overexpression in these patients and two biomarkers' relationship is not fully understood.

In this chapter, we sought to provide a comprehensive overview of the biological role of HER2 biomarker and current testing guidelines in breast and gastric cancer, in association with genetic *CDH1* alterations.

M. Ivanova
Division of Pathology, IEO, European Institute of Oncology IRCCS, Milan, Italy

E. Sajjadi · L. Zattoni · N. Fusco (✉)
Division of Pathology, IEO, European Institute of Oncology IRCCS, Milan, Italy

Department of Oncology and Haemato-Oncology, University of Milano, Milan, Italy
e-mail: nicola.fusco@ieo.it

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_10

10.1 Introduction

Receptor tyrosine-protein kinase erbB-2, also known as human epidermal growth factor receptor-2 (HER2), is a transmembrane glycoprotein with tyrosine kinase activity involved in controlling epithelial cell growth and differentiation. Dimerization of the receptor leads to various signal pathways activation resulting in cell proliferation and tumorigenesis. Although being present and widely studied in breast cancers (BC) and gastric cancers (GC), HER2 alterations (amplification, overexpression, or other mutations) are present in a plethora of other cancer types (esophageal, colon, lung, uterine cervix, endometrial, ovary, bladder, head and neck, and other cancers).

HER2 alterations could potentially drive the activation of its downstream signaling pathway resulting in increased tumor cell proliferation, reduced apoptosis, and enhanced migration. HER receptors are essential for cell proliferation, differentiation, adhesion, and survival which are activated through a variety of signaling pathways [1–5].

The expression of HER2 marker has both prognostic and predictive meaning, where the development of HER2-targeted therapies has dramatically influenced treatment strategies and clinical outcomes in patients with HER2-positive breast and gastric/gastroesophageal cancers, while results in other cancer types haven't been impressive so far [2, 3, 5].

This chapter provides a comprehensive overview on the biology of HER2 biomarker in cancer cells, HER2 testing in breast and gastric cancer with *CDH1* germline mutations, and highlights differences between HER2 expression in breast and gastric and gastroesophageal cancer.

10.2 The Biological Nature of HER2

HER2 is a 185 kD transmembrane glycoprotein located at the long arm of human chromosome 17 (17q12). This protein is a member of the epidermal growth factor receptor family (EGFR/ErbB) tyrosine kinase family which includes HER1/Erb1, HER2/Erb2, HER3/Erb3, and HER4/Erb4. HER proteins are ubiquitously expressed in epithelial, mesenchymal, and neuronal normal cells and progenitors and share common structural features, including single subunit glycoproteins with an extracellular ligand-binding site, a transmembrane lipophilic segment, and an intracellular tyrosine kinase catalytic domain. Upon ligands binding to their extracellular domains, HER proteins become activated through auto- and cross-phosphorylation triggering asymmetric dimerization of kinase domains. This process subsequently leads to kinase activation and signal transduction through oncogenic pathways (phosphoinositide-3-kinase (PI3K)/AKT/mTOR and RAS/RAF/MEK/ERK pathways). Unlike the other family members, HER2 lacks a ligand-binding domain and can be activated upon heterodimerization with HER1 and HER3 receptors. HER2-containing heterodimers have a great ligand binding affinity and signaling potency hence are the preferred dimerization partner of choice among the HER

family members. Dimerization and subsequent phosphorylation initiate a variety of signaling pathways, including principally the mitogen-activated protein kinase (MAPK), and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), pathways which are mainly involved in survival, proliferation, and cell-cycle progression (Fig. 10.1). The particular feature that contrasts HER2 among other family members is its resistance to internalization and degradation, so it can remain on the cell surface for a prolonged period after its activation, although the mechanisms are not yet fully understood. It is clear, however, that these properties pinpoint the receptor's property of transforming cells [2, 5–12].

The activation of HER2 signaling is observed in approximately 20% of breast cancers (BC) as the result of overexpression, owing to *ERBB2* amplification or activating somatic mutations, of which the most common are missense mutations in the tyrosine kinase and extracellular domains or duplications/insertions in a small stretch within exon 20. However, missense mutations of HER2 do not show complete transforming potential in absence of other oncogenes to confer a fully transformed phenotype. Co-occurring mutations, for example, with *PIK3CA*, found in approximately 1/3 of *HER2*-mutant breast cancers, augment the tumor pathway activation and could represent a future direction for target therapy development. Other less common biomarkers, involved in the pathways, of possible targeted therapy options are MSI/MMR, TMB, and NTRK. Generally, *HER2* mutations are almost exclusively present in cancers without *HER2* amplification and are associated with lobular breast, gastric, endometrial, and lung cancers [2, 6–16].

HER2 amplification on one side is a factor of a poor prognosis, due to the effect on cell proliferation, migration, and invasion. But on the other hand, it offers the possibility of a targeted treatment approach [2, 6–17].

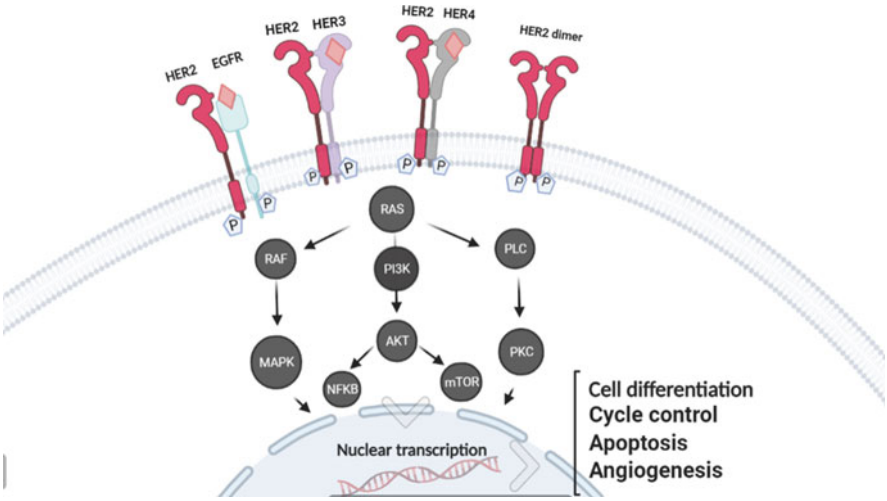


Fig. 10.1 HER2 signaling pathways and effects on cell cycle

HER2 expression and *HER2* gene amplification in gastric cancers (GC) account for up to 30% of tumors and are associated with a worse prognosis. Some studies indicate the role of HER2 in promoting epithelial-to-mesenchymal transition (EMT) in GC, and HER2 staining was found to correlate with tumor size, serosal invasion, lymph node metastases, and poor survival [2, 18–20].

It is known that HER2 plays an important role in neoplastic phenotype acquisition by gastroesophageal mucosa. In esophageal cancers, HER2 overexpression has been reported in 0–83%, with a higher rate in adenocarcinoma (10–83%) compared to squamous cell carcinoma (0–56%). Immunohistochemical assays (IHC) reveal HER2 expression in esophageal precancerous lesions with a gradually increasing rate within the dedifferentiation of paraneoplastic lesions. These results were confirmed by a dual-color silver in situ hybridization (SISH) probe, that demonstrated *HER2* amplification with its rates gradually rising from low-grade intraepithelial esophageal neoplasm to esophageal adenocarcinoma [2, 21]. The Barett's esophagus (BE), a known precursor of esophageal adenocarcinoma, is characterized by squamous epithelium displacement in gastroesophageal junction and intestinal metaplasia [2, 22, 23]. HER2 activation in esophageal epithelial cells was shown to be one of the key drivers of BE's neoplastic transformation, promoted by hyperinsulinemia and high C-peptide levels [24]. HER2 positivity in esophageal adenocarcinoma (EC) and adjacent Barett's esophagus (BE) has shown an association with reduced tumor aggressiveness, lower tumor grade, and improved patient survival [23, 25]. The HER2 expression has not been found prognostic in EC without adjacent BE; however, its heterogeneous expression was a predictor of worse cancer-specific survival in EC, and overexpression with *HER2* gene amplification in esophageal squamous cell carcinoma is associated with lower rates of survival [2, 23, 26–28].

Amplification or overexpression of HER2 occurs in approximately 15–30% of breast cancers and 10–30% of gastric/gastroesophageal cancers and serves as both prognostic and predictive biomarkers. Given that HER2 is a potent (proto)oncogene, these tumors are more aggressive than HER2-negative cancers [29]. Such tumors are associated with increased disease recurrence and poor survival outcomes if anti-HER2 treatment is not administered to the patients. HER2-targeted therapy has increased overall survival in HER2-positive breast and gastric cancers and has become the standard-of-care treatment for this group of patients [2].

10.3 HER2 Testing: Current Testing Guidelines

10.3.1 Breast Cancer

HER2 testing is currently recommended for all newly diagnosed breast cancers and possible retesting is considered in some cases of tumor progression or neoadjuvant treatment [4, 30, 31].

The HER2 testing relies on immunohistochemistry (IHC) scoring and/or gene amplification at in situ hybridization (ISH). IHC identifies and describes HER2

protein expression pattern and its intensity on the membrane of cancer cells, while ISH detects the presence of the gene amplification using HER2 and CEP17 probes. The first part of HER2 testing requires IHC analysis on a formalin-fixed paraffin-embedded tissue block involving expression score evaluation (range from 0 to 3+). According to the American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP) updated guidelines (2018), the HER2 test positivity is defined as protein overexpression (score 3+, complete strongly positive membrane staining in >10% of tumor cells) at IHC and/or gene amplification ISH. The score of 2+ (weak to moderate membrane staining in >10% of tumor cells) is considered equivocal and requires ISH as a reflex test, which requires an average HER2 copy number ≥ 6.0 signals/cell to consider the sample as HER2 positive. The IHC HER2 score of 0 and 1+ (negative or weak membrane staining in >10% of tumor cells), and HER2 2+ without gene amplification are considered HER2-negative. The 2018 ASCO/CAP guidelines summary of HER2 evaluation in breast cancers by ISH is presented in Table 10.1 [5, 31–33].

It is known that equivocal HER2 status may be associated with higher tumor grade, larger size, lymph node metastasis, and with lower overall and disease-free survival.

Nevertheless, recently introduced novel anti-HER2 therapies led to questioning the traditional scoring system, as it has been shown that patients with low HER2 breast cancer expression (score 1+/2+ without gene amplification), or who result negative for HER2 ISH/IHC assessment, may be considered "HER2-low" and benefit from HER2 antibody-drug conjugates (ADC). Furthermore, the advancement of HER2 targeting therapies and positive results of novel ADC versions have led to the "HER2-ultra low" (score 0 with incomplete faint staining in ≤10% of tumor cells) concept introduction, where patients may also receive a potential benefit of ADCs administration [4, 31, 32, 34–37]. New ADC versions as trastuzumab-duocaramzine (SYD-985) and trastuzumab-deruxtecan (DS-8201) have demonstrated favorable outcomes in preliminary data in metastatic breast cancer with low HER2 expression Although the ASCO/CAP guidelines have clearly defined criteria for HER2 status assessment in breast cancer, HER2-low expression formally has not been defined. The data from ongoing trials may provide more information on the correlation between response to treatment and HER2 expression patterns as well as with possible other response predictors [4, 35, 36, 38].

Overall, IHC and ISH probes show excellent correlation and a high concordance rate between core biopsy and excision statement. However, one should be aware of HER2 tumor heterogeneity in BC which occurs at variable frequencies (1–34%) in "mosaic"/"clustered" or "scattered" patterns, and could be the reason of HER2 equivocal status by ISH and/or IHC. Hormone receptor status also has its impact on the intrinsic molecular subtypes distribution of HER2-positive carcinomas [2, 16, 21, 34].

In breast cancers, the Expert Panel recommends that laboratories using single-probe ISH assays include concomitant IHC review as part of the interpretation of all single-probe ISH assay results [32]. This could raise the question if the current HER2 assessment has full compliance with HER2 signaling dysfunction. One

Table 10.1 The 2018 ASCO/CAP guidelines summary of HER2 evaluation in breast cancer by ISH. AMP, amplified; IHC, immunohistochemistry; ISH, in situ hybridization

Assay	Method	Result	Additional work-up	Final HER2 assignment
Dual-probe ISH	ISH algorithm	**Group 1** HER2/CEP17 ratio ≥ **2.0** and average HER2 copy number ≥ **4.0** signals/cell	/	AMP/positive
		Group 2 HER2/CEP17 ratio ≥ **2.0** and average HER2 copy number < **4.0** signals/cell	Concurrent IHC score **0/1+**	NOT AMP/negative with comment
			Concurrent IHC score **2+**, recount ISH: Preliminary result confirmed	NOT AMP/negative with comment
			Concurrent IHC score **2+**, recount ISH: Other ISH result	Result should be adjudicated per internal procedures
			Concurrent IHC score **3+**	AMP/positive
		Group 3 HER2/CEP17 ratio < **2.0** and average HER2 copy number ≥ **6.0** signals/cell	Concurrent IHC score **0/1+**	NOT AMP/negative with comment
			Concurrent IHC score **2+**, recount ISH: Preliminary result confirmed	AMP/positive
			Concurrent IHC score **2+**, recount ISH: Other ISH result	Result should be adjudicated per internal procedures
			Concurrent IHC score **3+**	AMP/positive
		Group 4 HER2/CEP17 ratio < **2.0** and average HER2 copy number ≥ **4.0** and < **6.0** signals/cell	Concurrent IHC score **0/1+**	NOT AMP/negative with comment
			Concurrent IHC score **2+** (**HER2 double-equivocal**), recount ISH: Preliminary result confirmed	NOT AMP/negative with comment
			Concurrent IHC score **2+** (**HER2 double-equivocal**), recount ISH: Other ISH result	Result should be adjudicated per internal procedures
			Concurrent IHC score **3+**	AMP/positive
		Group 5 HER2/CEP17 ratio < **2.0** and average HER2 copy	/	NOT AMP/negative

(continued)

Table 10.1 (continued)

Assay	Method	Result	Additional work-up	Final HER2 assignment
		number < **4.0** signals/cell		
		Group 5 HER2/CEP17 ratio < **2.0** and average HER2 copy number < **4.0** signals/cell	/	NOT AMP/negative
Single-probe ISH	HER2 **copy number**	Average HER2 copy number < **4.0** signals/cell	/	NOT AMP/negative
		Average HER2 copy number ≥ **4.0** and < **6.0** signals/cell	Concurrent IHC score **0/1+** and/or concurrent dual-probe ISH **group 5**	NOT AMP/negative
			Concurrent IHC score **2+**	Perform dual-probe ISH for final result
			Concurrent IHC score **3+** and/or concurrent dual-probe ISH **group 1**	AMP/positive
		Average HER2 copy number ≥ **6.0** signals/cell	/	AMP/positive

should also keep in mind that HER2-negative tumors tested in core biopsy samples may undergo a change of HER2 status due to intra-tumor heterogeneity, which is often the main reason of IHC and ISH assays discordance and some authors advocate simultaneous IHC and ISH conduction. Current ASCO/CAP guidelines (2018) recommend repeating HER2 testing on the resection specimen in case of HER2-negative or equivocal core biopsy result if the biopsy material was scarce, the tumor is of a high grade or presents unseen on biopsy morphological features. All the above guidelines apply for invasive breast cancer assessment regardless of their ductal or lobular morphology [4, 34, 36, 39–43].

10.3.2 Gastric Cancer

HER2 overexpression and/or amplification in patients with gastric cancer (GC) has been reported from 10 to 30%. A large number of studies indicate HER2 as a negative prognostic factor in GC, defining aggressive tumor behavior and higher recurrence frequency, but also responsiveness to HER2-targeted therapy [2, 11, 44].

Although the incidence of HER2 positivity in patients with BC and GC is similar, there are some important differences in tumor expression assessment to consider. In

contrast to BC, around 90% of HER2-positive GCc are reported to harbor HER2 overexpression in less than 5% [2, 6, 17, 45, 46].

Similar to BC, current ASCO/CAP and National Comprehensive Cancer Network (NCCN) guidelines (2016, 2017) consider HER2 testing using IHC and ISH assays. HER2 3+ score is considered positive and it is recommended that equivocal (2+) HER2 score should be examined by ISH, while NCCN suggests that any HER2 staining less than 3+ should be followed by ISH testing. HER2 2+ (equivocal) score is considered as weak to moderate incomplete (basolateral) membranous staining in >10% of tumor cells in the resection or only one cohesive cluster of >5 cells in biopsies). Score 1+ is considered as faint membranous reactivity in >10% of tumor cells in resection specimen or one cohesive cluster of >5 cells in biopsies. Score 0 is accounted as no or weak membranous reactivity in <10% of cells (resection) or one cohesive cluster of >5 cells in the biopsy. Scores 1+ and 0 are referred to HER2 negativity.

In GC, only the equivocal (2+ by IHC) samples are considered for ISH which accounts as positive at *HER2*:CEP17 ratio ≥2. Specimens with an IHC score of 0 or 1+ are considered negative and do not warrant further testing. The concordance between IHC 3+ and ISH positivity, reported in the literature, is very high (>90%).

The heterogeneity of HER2 expression is a common feature in HER2-positive GC, most often detected in the mixed histological type. Unlike BC, a complete membrane staining is not required for the gastric tumor to be considered as HER2-positive. This is partially explained by the histology of GC itself, where gland formation and mucin production may result in incomplete, basolateral, or lateral patterns of HER2 staining. Another common explanation is related to bacterial effects of *Helicobacter pylori*, which is a common agent in GC etiology. However, the heterogeneity of HER2 genotype may lead to discrepancies between IHC and ISH results and has been observed in about 5% of patients. The heterogeneity of HER2 genotype in gastric cancer may lead to discrepancies of the results between IHC and ISH. Ideally, the same tissue block should be used for ISH and IHC assay. The molecular heterogeneity of GC is referred to intratumor variation in genotype or gene expression resulting in focal positivity by IHC or ISH. False positivity also may be observed in areas of intestinal metaplasia or high-grade dysplasia, adjacent to ulcer or necrotic sites, and not taken into account [2, 17, 44–46].

One of the biggest trials reporting HER2 expression in GC, Trastuzumab for GAstric cancer (ToGA), analyzed 3807 stomach and gastroesophageal junction cancer patients' specimens using both ICH and ISH assays. The HER2 positivity rate was 22.1% and those patients were randomized into two groups: chemotherapy only or chemotherapy with trastuzumab administration.

The results demonstrated the high efficacy of HER2 targeting therapies in HER2-positive GC or gastroesophageal junction cancer patients. The median overall survival and health-related quality of life were significantly higher for patients receiving trastuzumab and chemotherapy compared to chemotherapy alone. Even though the most benefit was derived by patients with HER2 3+/2+ ISH amplified score, further addition of trastuzumab didn't show any advantage upon further follow-up. Patients with IHC scores 0 and 1+ have shown *HER2* ISH positivity in

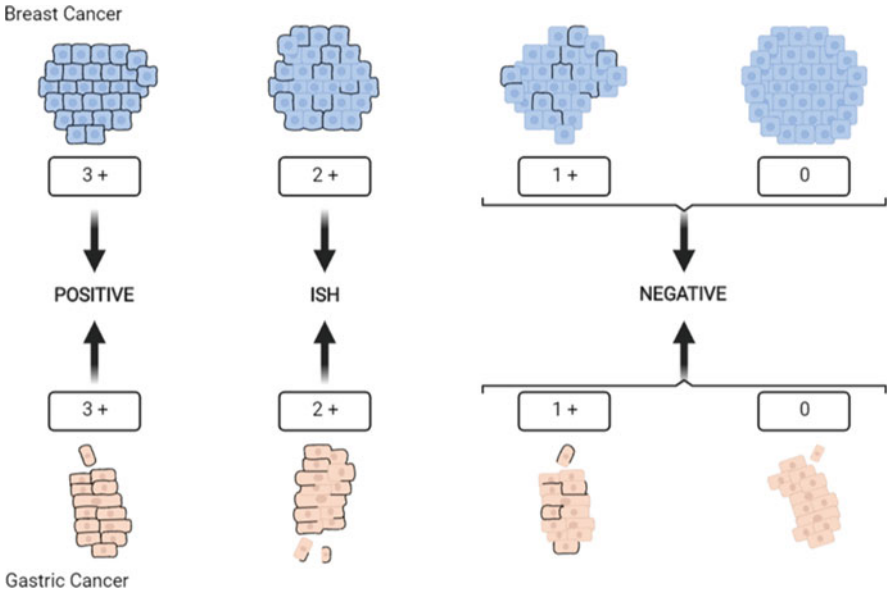


Fig. 10.2 HER2 immunohistochemical assessment patterns in breast and gastric cancer according to current ASCO/CAP guidelines (2018)

14–24% of cases and did not significantly benefit from the addition of trastuzumab, and it has been stated that ISH positivity alone does not correlate with response to trastuzumab therapy. Overall, the data shown support that the determination of HER2 status in patients with resectable GC is not useful in survival prognosis assessment. However, GC patients with good performance status who would be candidates for systemic therapy should undergo HER2 testing and be offered trastuzumab if tested positive. Nowadays, the focus on HER2 expression/amplification status alone is not able to capture the underlying mechanisms of disease progression and resistance [6, 11, 17, 44, 46–50] (Fig. 10.2).

10.4 HER2 Testing in Hereditary Breast and Gastric Cancers

Hereditary diffuse gastric cancer syndrome (HDGS) is linked to *CDH1* gene alterations.

CDH1 is located on chromosome 16q22.1 and encodes the cell-to-cell adhesion protein E-cadherin, and its germline variants are playing a crucial role in HDGS with a high prevalence of diffuse gastric cancer (DGC) and lobular breast cancer (LBC) [51, 52].

E-cadherin is a protein responsible for differentiated epithelium stability maintenance through intracellular adhesion complexes. The main role of E-cadherin in tumorigenesis thus is cell invasion suppression, deregulating infiltrative and

metastatic ability of the tumor triggered by loss of cells adhesion and increased cell motility acquisition. Some authors link loss of E-cadherin with epithelial-to-mesenchymal transition phenomena, which enhances tumor metastatic potential. Loss of E-cadherin is also associated with activation of EGFR through tumorigenic RAS/RAF/MEK, FAK/c-Src, and PI3K/AKT/mTOR pathways, which drive cell proliferation and motility [53–55].

Somatic *CDH1* alterations are associated with a poor prognosis in DGC and LBC. The *CDH1* gene mutations occur most frequently as a missense type and it is known that *CDH1* mutation carriers have a cumulative risk of 70–79% of HGCS for men and 56% for women by age of 80, and a cumulative risk of LBC 42–46% by age of 80 years for women. The role of this gene is not yet well assessed in other cancer types; however, the risk of occurrence of colorectal and signet-ring cell gastric carcinomas, prostate, ovarian, thyroid, and tongue cancers in *CDH1* variants and mutation carriers is noted. The studies assessing the possible role of *CDH1* mutations are ongoing [51–62].

10.4.1 Hereditary Lobular Breast Cancer

Breast cancer (BC) is one of the most common female cancers in the world and nearly 20% of BCs express high level of human epidermal growth factor receptor 2 (HER2). HER2 is involved in the growth of cancer cells and determines the aggressive clinical course of BC. Overexpression of HER2 in BC occurs mainly due to *ERBB2(HER2)* gene amplification and one of the established therapeutic targets in HER2-positive BC cancer patients is anti-HER2 therapy. There is a variety of agents as trastuzumab, pertuzumab, lapatinib, neratinib, and trastuzumab emtansine (T-DM1), approved for the treatment of HER2-positive BC. The notable effect of trastuzumab addition to chemotherapy has dramatically improved overall survival of those patients [4, 11, 35, 36].

Rare *HER2* mutations typically occur in absence of *HER2* amplification (<2%) and continue raising attention due to their potential driver role in *HER2* nonamplified tumors. They are most frequently found in invasive lobular breast cancer (LBC), especially high-grade, accounting for 5–15% of total breast cancers, and characterized by estrogen receptor (ER) positivity, frequent HER2 negativity, and loss of E-cadherin function. One of the main reasons of E-cadherin loss are *CDH1* mutations, observed in up to 83% of LBCs and some of these patients may simultaneously carry both *CDH1* and *HER2* mutations. The role of E-cadherin loss in case of LBC promotes loss of cell-to-cell adhesion, increased cell proliferation, and lobular hyperplasia, where deregulated cells create a lobular intraepithelial neoplasia pattern with subsequent basement membrane disruption [33, 52, 63–67].

The female *CDH1* mutation carriers are at a high risk of LBC and HGDC development, although the frequency of a germline CDH1 mutation is very low (~1%) in women with early-onset or familial LBC without a family history of GC. The risk of developing LBC in absence of HDGC history is unknown. However, recent studies demonstrate that LBC may be the first manifestation of HGDC

syndrome even in absence of a family history of DGC. It was also supposed that familiar LBC with *CDH1* germline mutations may be an independent inherited syndrome, therefore the concept of hereditary lobular breast cancer (HLBC) has been proposed. Hereditary basis of LBC has been supported by a high frequency of bilateral disease and overall familiality in population studies. Regarding LBC presenting CDH1 germline pathogenic alteration, no information is available about overall survival and it does not seem to have an impact in prognosis [33, 52, 57, 59, 68].

Pathologic assessment of BC patients currently requires evaluation of tumor size, lymph node status, histological grade, estrogen receptor (ER), progesterone receptor (PR), and HER2 expression. These factors, however, do not predict BC prognosis accurately. The pathology of LBC usually represents ER/PR-positive and HER2-negative phenotype. In this context it is plausible to pinpoint that *CDH1* hypermethylation is strongly associated with ER-negative and HER2-negative BC and aggressive tumor behavior. These findings reflect the knowledge of HER2 belonging to the family of EGFR, whose pathway is activated by loss of E-cadherin. Loss of E-cadherin is associated with HER2-negativity, lower tumor differentiation, and bigger size. The useful feature in diagnosing these patients is thus the IHC E-cadherin expression, where its loss is associated with worse overall survival. The role of HER2 in relapsed LBC has also been suggested, while some other studies demonstrate no association between *CDH1* and *HER2* genes even though they are both cancer drivers [5, 33, 61, 62, 67, 69–71].

Comprehensive genomic profiling of relapsed *CDH1*-mutated ILC revealed actionable genomic alterations with a high incidence of *HER2* mutations. It has been shown that simultaneous presence of *CDH1* and *HER2* mutations lead to a worse prognosis, nevertheless, the effect is not completely clear. LBCs are considered majorly unresponsive to tyrosine kinase inhibitors and chemotherapy; however, identification of *HER2* mutations could permit using targeted therapy as trastuzumab. Current reports illustrate the case of metastatic HER2-nonamplified breast cancer with identified *HER2* and *CDH1* mutations in retropectoral muscle metastases who had impressive clinical results with *HER2*-targeting therapy laptanamib. These data warrant *HER2* status assessment in all ILC cases with *CDH1* aberrations [5, 33, 61, 62, 64, 67–69, 72].

There are currently no widely used criteria for *CDH1* genetic screening of LBC predisposition without gastric cancer association; however, an international expert panel on hereditary LBC has developed suggested criteria for *CDH1* testing [33, 61].

In light of ongoing studies, current HER2 scoring, probably, should be reconsidered, issuing new guidelines in HER2 molecular analysis in LBC patients with confirmed *CDH1* mutations, to provide clinically useful information to improve patients' management, clinical outcomes, and prognosis, as the *HER2* aberrations revealed may give consideration to HER2-targeted therapy even in absence of HER2 amplification [5, 33, 61, 62, 67–69, 72].

10.4.2 Hereditary Diffuse Gastric Cancer

There are two major subtypes of gastric cancer (GC) in the world: diffuse and intestinal GC, and both of them are associated with a spectrum of molecular, genetic, and epigenetic abnormalities. Molecular profiling has provided a new framework for GC classifications by molecular abnormalities, proposed by The Cancer Genome Atlas (TCGA) Research Network and the Asian Cancer Research Group (ACRG), defining four GC subtypes: EBV-positive, microsatellite-unstable, genomically stable, and chromosomally unstable [73–76].

The *ERBB2(HER2)* gene amplification assays identify patients susceptible to targeted anti-HER2 therapy. HER2 is overexpressed in up to 20% of gastric and gastroesophageal junction cancers and the assessment of HER2 in these patients is mandatory. The Trastuzumab for GAstric cancer (ToGA) trial has demonstrated that addition of trastuzumab to chemotherapy in HER2-positive GC improves overall survival, and has become a standard treatment. Interestingly, other trials involving pertuzumab, lapatinib, and T-DM1 have failed to provide significant improvements in the outcomes of patients with HER2-positive GC [11, 17, 21, 77, 78].

In overall GC, the expression of HER2 is higher in intestinal vs. diffuse type (31.8% vs. 6.1%). While intestinal-type GC maintains typical membranous staining, diffuse-type GC may represent membranous and cytoplasmic staining, probably due to extracellular domain shedding [78, 79].

Diffuse type GC (DGC) accounts for about 10% of GC and is known for its aggressive clinical behavior and familial aggregation. Hereditary diffuse gastric cancer (HDGC) is estimated to be $<1\%$ of all GC cases. HDGC is frequently associated with E-cadherin coding gene *CDH1* alterations and up to 50% of sporadic diffuse gastric cancers (DGC) have somatically inactive E-cadherin mutations. Clinical studies reveal that GC patients with *CDH1* mutations are characterized by tumor aggressiveness, have shorter survival times and overall worse prognosis. All subjects with a family history of GC, suspected to HGDC, undergo DNA *CDH1* testing and are recommended to undergo the prophylactic gastrectomy if tested positive [52, 53, 56, 68, 80–82].

The relation between HER2 overexpression and CDH1 alterations and their effect on patient survival is unknown. Histologically, DGC lacks the expression of adhesion molecules and poor differentiation. While *CDH1* mutations can be reflected with CDH1 protein loss by IHC and are detected in 40% of DGC, its relationship with *HER2* is not well studied, although DGC with *CDH1* mutations were reported to have a lower rate of HER2 positivity compared to intestinal-type GC. Indeed, one of the largest cohort studies of *CDH1*-mutated cancers aiming to discover novel therapeutic studies was conducted in 1596 patients and did not reveal a significant rate of HER2 amplification. The HER2 enrichment in DGS is analogous to those of LBC, therefore the female preponderance in DGC raises the concern for misdiagnosed LBC [56, 80, 82–86].

It has been shown that overall in GC patients, higher E-cadherin levels correlate with HER2 positivity and show better overall survival when maintaining a high E-cadherin expression and HER2-positive status compared to HER2-negative. Poor

survival in HER2-positive GC is related to loss of E-cadherin, mediated by Wnt/β-catenin pathway, where the disruption of the binding of β-catenin to E-cadherin leads to loss of the latter from the cell surface promoting tumor growth. HER2 overexpression is associated with Wnt-β-catenin activation and with an increase in CDH1 mRNA production. The *HER2* targeting therapy is reported to reduce the *CDH1* mRNA in GC, leading to reduction of E-cadherin release and Wnt/β-catenin activation [11, 83, 87].

The loss of E-cadherin is also known as a hallmark of epithelial-to-mesenchymal transition (EMT), which is related to cancer metastatic potential, by changing cancer cells' phenotype, so better understanding of the relationship between E-cadherin and HER2 overexpression may be useful to uncover the pathogenesis of GCs [56, 60, 83, 88].

By now, the choice of treatment for GC and DGC patients is only guided by validated biomarkers as *HER2* and microsatellite instability/PD-L1. Tumor heterogeneity in GC may lead to loss of *HER2* signaling after trastuzumab therapy, which raises questions about the marker's utility and requires more studies. Novel findings support a possible functional role of E-cadherin in response to anti-HER2 treatment; however, the role of *CDH1* mutations in this context requires more studies to define molecular interactions between *HER2* and E-cadherin and their role as predictive factors for targeted therapy [81, 83, 87].

References

1. Reichelt U, Duesedau P, Tsourlakis MC, Quaas A, Link BC, Schurr PG et al (2007) Frequent homogeneous HER-2 amplification in primary and metastatic adenocarcinoma of the esophagus. Mod Pathol 20(1):120–129
2. Iqbal N, Iqbal N (2014) Human epidermal growth factor receptor 2 (HER2) in cancers: overexpression and therapeutic implications. Mol Biol Int 2014:852748
3. Miglietta F, Griguolo G, Bottosso M, Giarratano T, Lo Mele M, Fassan M et al (2021) Evolution of HER2-low expression from primary to recurrent breast cancer. NPJ Breast Cancer 7(1):137
4. Venetis K, Crimini E, Sajjadi E, Corti C, Guerini-Rocco E, Viale G et al (2022) HER2 low, ultra-low, and novel complementary biomarkers: expanding the spectrum of HER2 positivity in breast cancer. Front Mol Biosci 9:834651
5. He L, Araj E, Peng Y (2021) HER2 positive and HER2 negative classical type invasive lobular carcinomas: comparison of clinicopathologic features. Curr Oncol 28(3):1608
6. Fusco N, Bosari S (2016) HER2 aberrations and heterogeneity in cancers of the digestive system: implications for pathologists and gastroenterologists. World J Gastroenterol 22(35): 7926–7937
7. Aoki M, Iwasa S, Boku N (2021) Trastuzumab deruxtecan for the treatment of HER2-positive advanced gastric cancer: a clinical perspective. Gastric Cancer 24(3):567–576
8. Hanker AB, Brown BP, Meiler J, Marín A, Jayanthan HS, Ye D et al (2021) Co-occurring gain-of-function mutations in HER2 and HER3 modulate HER2/HER3 activation, oncogenesis, and HER2 inhibitor sensitivity. Cancer Cell 39(8):1099–114.e8
9. Arteaga CL, Engelman JA (2014) ERBB receptors: from oncogene discovery to basic science to mechanism-based cancer therapeutics. Cancer Cell 25(3):282–303
10. Jeong J, Kim W, Kim LK, VanHouten J, Wysolmerski JJ (2017) HER2 signaling regulates HER2 localization and membrane retention. PLoS One 12(4):e0174849

11. Oh DY, Bang YJ (2020) HER2-targeted therapies - a role beyond breast cancer. Nat Rev Clin Oncol 17(1):33–48
12. Pollock NI, Grandis JR (2015) HER2 as a therapeutic target in head and neck squamous cell carcinoma. Clin Cancer Res 21(3):526–533
13. Criscitiello C, Marra A, Curigliano G (2021) PIK3CA mutation assessment in HR+/HER2− metastatic breast cancer: overview for oncology clinical practice. J Mol Pathol 2(1)
14. Fusco N, Malapelle U, Fassan M, Marchiò C, Buglioni S, Zupo S et al (2021) PIK3CA mutations as a molecular target for hormone receptor-positive, HER2-negative metastatic breast cancer. Front Oncol 11
15. Najjar S, Allison KH (2022) Updates on breast biomarkers. Virchows Arch 480(1):163–176
16. Marchiò C, Annaratone L, Marques A, Casorzo L, Berrino E, Sapino A (2021) Evolving concepts in HER2 evaluation in breast cancer: heterogeneity, HER2-low carcinomas and beyond. Semin Cancer Biol 72:123–135
17. Bang Y-J, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A et al (2010) Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 376(9742):687–697
18. Yonemura Y, Ninomiya I, Yamaguchi A, Fushida S, Kimura H, Ohoyama S et al (1991) Evaluation of immunoreactivity for erbB-2 protein as a marker of poor short term prognosis in gastric cancer. Cancer Res 51(3):1034–1038
19. Nakajima M, Sawada H, Yamada Y, Watanabe A, Tatsumi M, Yamashita J et al (1999) The prognostic significance of amplification and overexpression of c-met and c-erb B-2 in human gastric carcinomas. Cancer 85(9):1894–1902
20. Liu Y, Chen L, Jiang D, Luan L, Huang J, Hou Y et al (2021) HER2 promotes epithelial-mesenchymal transition through regulating osteopontin in gastric cancer. Pathol – Res Pract 227:153643
21. Fassan M, Mastracci L, Grillo F, Zagonel V, Bruno S, Battaglia G et al (2012) Early HER2 dysregulation in gastric and oesophageal carcinogenesis. Histopathology 61(5):769–776
22. Falk GW (2002) Barrett's esophagus. Gastroenterology 122(6):1569–1591
23. Nagaraja V, Shaw N, Morey AL, Cox MR, Eslick GD (2016) HER2 expression in oesophageal carcinoma and Barrett's oesophagus associated adenocarcinoma: an Australian study. Eur J Surg Oncol 42(1):140–148
24. Arcidiacono D, Antonello A, Fassan M, Nucci D, Morbin T, Agostini M et al (2017) Insulin promotes HER2 signaling activation during Barrett's esophagus carcinogenesis. Dig Liver Dis 49(6):630–638
25. Yoon HH, Shi Q, Sukov WR, Wiktor AE, Khan M, Sattler CA et al (2012) Association of HER2/ErbB2 expression and gene amplification with pathologic features and prognosis in esophageal adenocarcinomas. Clin Cancer Res 18(2):546–554
26. Yoon HH, Shi Q, Sukov WR, Lewis MA, Sattler CA, Wiktor AE et al (2012) Adverse prognostic impact of intratumor heterogeneous HER2 gene amplification in patients with esophageal adenocarcinoma. J Clin Oncol 30(32):3932–3938
27. Zhan N, Dong WG, Tang YF, Wang ZS, Xiong CL (2012) Analysis of HER2 gene amplification and protein expression in esophageal squamous cell carcinoma. Med Oncol 29(2):933–940
28. Gowryshankar A, Nagaraja V, Eslick GD (2014) HER2 status in Barrett's esophagus & esophageal cancer: a meta analysis. J Gastrointest Oncol 5(1):25–35
29. Hamilton E, Shastry M, Shiller SM, Ren R (2021) Targeting HER2 heterogeneity in breast cancer. Cancer Treat Rev 100:102286
30. Viale G, Fusco N (2021) Pathology after neoadjuvant treatment – how to assess residual disease. Breast 56:S13
31. Ahn S, Woo JW, Lee K, Park SY (2020) HER2 status in breast cancer: changes in guidelines and complicating factors for interpretation. J Pathol Transl Med 54(1):34–44

32. Wolff AC, Hammond MEH, Allison KH, Harvey BE, McShane LM, Dowsett M (2018) HER2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline focused update summary. J Oncol Pract 14(7):437–441
33. WHO Classification of Tumours Editorial Board (2019) WHO Classification of Breast Tumours: WHO Classification of Tumours, vol 2. World Health Organization
34. Zhang H, Moisini I, Ajabnoor RM, Turner BM, Hicks DG (2020) Applying the new guidelines of HER2 testing in breast cancer. Curr Oncol Rep 22(5):51
35. Tarantino P, Hamilton E, Tolaney SM, Cortes J, Morganti S, Ferraro E et al (2020) HER2-low breast cancer: pathological and clinical landscape. J Clin Oncol 38(17):1951–1962
36. Tarantino P, Gandini S, Nicolò E, Trillo P, Giugliano F, Zagami P et al (2022) Evolution of low HER2 expression between early and advanced-stage breast cancer. Eur J Cancer 163:35–43
37. Fusco N, Rocco EG, Del Conte C, Pellegrini C, Bulfamante G, Di Nuovo F et al (2013) HER2 in gastric cancer: a digital image analysis in pre-neoplastic, primary and metastatic lesions. Mod Pathol 26(6):816–824
38. Banerji U, van Herpen CML, Saura C, Thistlethwaite F, Lord S, Moreno V et al (2019) Trastuzumab duocarmazine in locally advanced and metastatic solid tumours and HER2-expressing breast cancer: a phase 1 dose-escalation and dose-expansion study. Lancet Oncol 20(8):1124–1135
39. MacNeil IA, Burns DJ, Rich BE, Soltani SM, Kharbush S, Osterhaus NG et al (2020) New HER2-negative breast cancer subtype responsive to anti-HER2 therapy identified. J Cancer Res Clin Oncol 146(3):605–619
40. Zhang H, Katerji H, Turner BM, Audeh W, Hicks DG (2022) HER2-low breast cancers: incidence, HER2 staining patterns, clinicopathologic features, MammaPrint and BluePrint genomic profiles. Mod Pathol 35:1075
41. Nitta H, Kelly BD, Allred C, Jewell S, Banks P, Dennis E et al (2016) The assessment of HER2 status in breast cancer: the past, the present, and the future. Pathol Int 66(6):313–324
42. Jensen SG, Thomas PE, Christensen IJ, Balslev E, Hansen A, Høgdall E (2020) Evaluation of analytical accuracy of HER2 status in patients with breast cancer: comparison of HER2 GPA with HER2 IHC and HER2 FISH. APMIS 128(11):573–582
43. Gordian-Arroyo AM, Zynger DL, Tozbikian GH (2019) Impact of the 2018 ASCO/CAP HER2 guideline focused update. Am J Clin Pathol 152(1):17–26
44. Abrahao-Machado LF, Scapulatempo-Neto C (2016) HER2 testing in gastric cancer: an update. World J Gastroenterol 22(19):4619–4625
45. Setia N, Ahn S, Han HS, Park DY, Lauwers GY (2019) Predictive value of WHO classification for PD-L1 and Her2/Neu expression and distinct associations with protein expression based classification in gastric carcinoma. Hum Pathol 94:64–70
46. Bartley AN, Washington MK, Colasacco C, Ventura CB, Ismaila N, Benson AB 3rd et al (2017) HER2 testing and clinical decision making in gastroesophageal adenocarcinoma: guideline from the College of American Pathologists, American Society for Clinical Pathology, and the American Society of Clinical Oncology. J Clin Oncol 35(4):446–464
47. Satoh T, Bang YJ, Gotovkin EA, Hamamoto Y, Kang YK, Moiseyenko VM et al (2014) Quality of life in the trastuzumab for gastric cancer trial. Oncologist 19(7):712–719
48. Kurokawa Y, Matsuura N, Kimura Y, Adachi S, Fujita J, Imamura H et al (2015) Multicenter large-scale study of prognostic impact of HER2 expression in patients with resectable gastric cancer. Gastric Cancer 18(4):691–697
49. Janjigian YY, Werner D, Pauligk C, Steinmetz K, Kelsen DP, Jäger E et al (2012) Prognosis of metastatic gastric and gastroesophageal junction cancer by HER2 status: a European and USA international collaborative analysis. Ann Oncol 23(10):2656–2662
50. Van Cutsem E, Kang YK, Shen L, Lordick F, Ohtsu A, Satoh T et al (2009) 7BA Trastuzumab added to standard chemotherapy (CT) as first-line treatment in human epidermal growth factor receptor 2 (HER2)-positive advanced gastric cancer (GC): efficacy and safety results from the phase III ToGA trial. Eur J Cancer Suppl 7(3):7

51. Adib E, El Zarif T, Nassar AH, Akl EW, Abou Alaiwi S, Mouhieddine TH et al (2022) CDH1 germline variants are enriched in patients with colorectal cancer, gastric cancer, and breast cancer. Br J Cancer 126(5):797–803
52. Massari G, Magnoni F, Favia G, Peradze N, Veronesi P, La Vecchia C et al (2021) Frequency of CDH1 germline mutations in non-gastric cancers. Cancers (Basel) 13(10)
53. Zhao H, Hu H, Chen B, Xu W, Zhao J, Huang C et al (2021) Overview on the role of E-cadherin in gastric cancer: dysregulation and clinical implications. Front Mol Biosci 8:689139
54. Shenoy S (2019) CDH1 (E-cadherin) mutation and gastric cancer: genetics, molecular mechanisms and guidelines for management. Cancer Manag Res 11:10477–10486
55. Han T, Jiang S, Zheng H, Yin Q, Xie M, Little MR et al (2019) Interplay between c-Src and the APC/C co-activator Cdh1 regulates mammary tumorigenesis. Nat Commun 10(1):3716
56. Hansford S, Kaurah P, Li-Chang H, Woo M, Senz J, Pinheiro H et al (2015) Hereditary diffuse gastric cancer syndrome: CDH1 mutations and beyond. JAMA Oncol 1(1):23–32
57. Schrader KA, Masciari S, Boyd N, Wiyrick S, Kaurah P, Senz J et al (2008) Hereditary diffuse gastric cancer: association with lobular breast cancer. Familial Cancer 7(1):73–82
58. (2021) Breast cancer risk genes — association analysis in more than 113,000 women. N Engl J Med 384(5):428–439
59. Luo W, Fedda F, Lynch P, Tan D (2018) CDH1 gene and Hereditary diffuse gastric cancer syndrome: molecular and histological alterations and implications for diagnosis and treatment. Front Pharmacol 9:1421
60. Hu MN, Hu SH, Zhang XW, Xiong SM, Deng H (2020) Overview on new progress of hereditary diffuse gastric cancer with CDH1 variants. Tumori 106(5):346–355
61. Corso G, Figueiredo J, De Angelis SP, Corso F, Girardi A, Pereira J et al (2020) E-cadherin deregulation in breast cancer. J Cell Mol Med 24(11):5930–5936
62. Huang R, Ding P, Yang F (2015) Clinicopathological significance and potential drug target of CDH1 in breast cancer: a meta-analysis and literature review. Drug Des Devel Ther 9:5277–5285
63. McCart Reed AE, Kutasovic JR, Lakhani SR, Simpson PT (2015) Invasive lobular carcinoma of the breast: morphology, biomarkers and 'omics. Breast Cancer Res 17(1):12
64. Grellety T, Soubeyran I, Robert J, Bonnefoi H, Italiano A (2016) A clinical case of invasive lobular breast carcinoma with ERBB2 and CDH1 mutations presenting a dramatic response to anti-HER2-directed therapy. Ann Oncol 27(1):199–200
65. Koboldt DC, Fulton RS, McLellan MD, Schmidt H, Kalicki-Veizer J, McMichael JF et al (2012) Comprehensive molecular portraits of human breast tumours. Nature 490(7418):61–70
66. Krystel-Whittemore M, Wen HY (2022) Update on HER2 expression in breast cancer. Diagn Histopathol 28(3):170–175
67. Corso G, Figueiredo J, La Vecchia C, Veronesi P, Pravettoni G, Macis D et al (2018) Hereditary lobular breast cancer with an emphasis on E-cadherin genetic defect. J Med Genet 55(7):431–441
68. Corso G, Veronesi P, Sacchini V, Galimberti V (2018) Prognosis and outcome in CDH1-mutant lobular breast cancer. Eur J Cancer Prev:27(3)
69. Pramod N, Nigam A, Basree M, Mawalkar R, Mehra S, Shinde N et al (2021) Comprehensive review of molecular mechanisms and clinical features of invasive lobular cancer. Oncologist 26(6):e943–ee53
70. Karray-Chouayekh S, Trifa F, Khabir A, Sellami-Boudawara T, Frikha M, Gargouri A et al (2012) Negative/low HER2 expression alone or combined with E-cadherin positivity is predictive of better prognosis in patients with breast carcinoma. Histol Histopathol 27(3):377–385
71. Corso G, Bonanni B, Veronesi P (2018) Tumor inactivation of E-cadherin: a new tool for breast cancer treatment? Ann Transl Med 6:S6
72. Ross JS, Wang K, Sheehan CE, Boguniewicz AB, Otto G, Downing SR et al (2013) Relapsed classic E-cadherin (CDH1)-mutated invasive lobular breast cancer shows a high frequency of HER2 (ERBB2) gene mutations. Clin Cancer Res 19(10):2668–2676

73. Padmanabhan N, Ushijima T, Tan P (2017) How to stomach an epigenetic insult: the gastric cancer epigenome. Nat Rev Gastroenterol Hepatol 14(8):467–478
74. Yamazawa S, Ushiku T, Shinozaki-Ushiku A, Hayashi A, Iwasaki A, Abe H et al (2017) Gastric cancer with primitive enterocyte phenotype: an aggressive subgroup of intestinal-type adenocarcinoma. Am J Surg Pathol 41(7):989–997
75. Comprehensive molecular characterization of gastric adenocarcinoma (2014) Nature 513(7517):202–209
76. Katoh H, Ishikawa S (2017) Genomic pathobiology of gastric carcinoma. Pathol Int 67(2): 63–71
77. Grillo F, Fassan M, Sarocchi F, Fiocca R, Mastracci L (2016) HER2 heterogeneity in gastric/gastroesophageal cancers: from benchside to practice. World J Gastroenterol 22(26):5879–5887
78. Van Cutsem E, Bang YJ, Feng-Yi F, Xu JM, Lee KW, Jiao SC et al (2015) HER2 screening data from ToGA: targeting HER2 in gastric and gastroesophageal junction cancer. Gastric Cancer 18(3):476–484
79. Pandey I, Misra V, Pandey AT, Verma A (2022) Expression of HER2/neu in gastric adenocarcinoma and its correlation with serum HER2/neu level and E-cadherin expression. Indian J Pathol Microbiol 65(1):35–41
80. Muzashvili T, Tutisani A, Chabradze G, Beridze N, Museridze N (2020) The study of the expression of CDH1, KI67, P53 and HER2 in diffuse gastric carcinoma. Georgian Med News 299:147–150
81. Smyth EC, Nilsson M, Grabsch HI, van Grieken NC, Lordick F (2020) Gastric cancer. Lancet 396(10251):635–648
82. Iyer P, Moslim M, Farma JM, Denlinger CS (2020) Diffuse gastric cancer: histologic, molecular, and genetic basis of disease. Transl Gastroenterol Hepatol 5:52
83. De Re V, Alessandrini L, Brisotto G, Caggiari L, De Zorzi M, Casarotto M et al (2022) HER2-CDH1 interaction via Wnt/B-catenin is associated with patients' survival in HER2-positive metastatic gastric adenocarcinoma. Cancers (Basel) 14(5)
84. Blair VR, McLeod M, Carneiro F, Coit DG, D'Addario JL, van Dieren JM et al (2020) Hereditary diffuse gastric cancer: updated clinical practice guidelines. Lancet Oncol 21(8): e386–ee97
85. Wang J, Xiu J, Arai H, Battaglin F, Poorman K, Tokunaga R et al (2019) 679PD - large-scale analysis of CDH1 mutations define a distinctive molecular subset in gastric cancer (GC). Ann Oncol 30:v258
86. Hemmerich A, Madison R, Klempner S, Sokol E, Severson E, Miller V et al (2019) O-006 - genomic profiling of diffuse gastric carcinoma (DGC). Ann Oncol 30:iv128
87. Caggiari L, Miolo G, Buonadonna A, Basile D, Santeufemia DA, Cossu A et al (2017) Characterizing metastatic HER2-positive gastric cancer at the CDH1 haplotype. Int J Mol Sci 19(1)
88. Fang C, Kang Y (2021) E-cadherin: context-dependent functions of a quintessential epithelial marker in metastasis. Cancer Res 81(23):5800–5802

Pathology and Somatic Alterations in Hereditary Lobular Breast Cancers

11

Francesca Maria Porta, Marta Cruz Blanco, Mariia Ivanova, Nicola Fusco, and Elena Guerini-Rocco

Abstract

The most frequent special histological type of breast cancer is represented by invasive lobular carcinoma (ILC), which makes up about 15% of all invasive breast carcinomas. The molecular signature of ILC is the dysregulation of E-cadherin due to *CDH1* abnormalities. Although *CDH1* germline mutations are very uncommon in women with early-onset and/or familial ILC, they are the most common detrimental non-BRCA mutations and are thought to be the origin of a significant fraction of lobular breast cancer. Since the morphology and immunophenotype of hereditary and non-hereditary ILCs are nearly identical, no specific histopathological findings can be used to distinguish between the two. High-throughput sequencing studies revealed that ILCs represent a separate entity at the genomic level. This chapter addresses the very important topic of ILC morpho-molecular characteristics in the setting of germline and/or somatic *CDH1* abnormalities.

11.1 Introduction

Invasive lobular carcinoma (ILC) is the most common special type of breast cancer and accounts for ~15% of invasive breast carcinomas [1]. Dysregulation of E-cadherin due to *CDH1* aberrations is considered the molecular hallmark of ILC

F. M. Porta · N. Fusco · E. Guerini-Rocco (✉)
Division of Pathology, IEO, European Institute of Oncology IRCCS, Milan, Italy

Department of Oncology and Haemato-Oncology, University of Milan, Milan, Italy
e-mail: elena.guerinirocco@ieo.it; elena.guerini@unimi.it

M. C. Blanco · M. Ivanova
Division of Pathology, IEO, European Institute of Oncology IRCCS, Milan, Italy

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_11

[2, 3]. Although the frequency of *CDH1* germline mutations is very low (~1%) in women with early-onset or familial ILC, these mutations represent the most frequent deleterious non-BRCA mutations, and they are considered founder genetic events in a substantial proportion of lobular breast cancer [4–6]. No specific histopathological features can help discriminate between hereditary and non-hereditary ILCs because their morphology and immunophenotype are substantially identical [7]. However, ILCs display peculiar clinic-pathologic characteristics as compared to other breast cancer histotypes [1]. Moreover, high-throughput sequencing analyses showed that ILCs also represent a distinct entity at the genomic level [2, 3, 8, 9]. This chapter provides a comprehensive overview of the morpho-molecular characteristics of ILC in the context of germline and/or somatic CDH1 aberrations.

11.2 Pathology of Lobular Breast Cancer

Individuals with ILC typically have a diagnosis at an older age and come to the physician's attention with larger tumors than patients with invasive breast cancer (IBC) of no special type [10]. Hereditary ILC is often bilateral and multicentric, appearing as ill-defined palpable mass(es) or widespread breast nodularities [11]. Classic ILC is composed of non-to-poorly cohesive small, roundish, monomorphic neoplastic elements, with uniform nuclei, inconspicuous nucleoli, and infrequent mitotic figures interspersed into a variably dense fibrous stroma arranged in loose or linear growth pattern. ILC exhibits a targetoid concentric distribution around ducts and lobules and is usually associated with little host reaction [1, 12–16].

It is possible to identify different ILC variants, including solid, alveolar, trabecular, tubule-lobular, signet ring cell, pleomorphic, and histiocytoid which differ from classical ILC in their morphologic characteristics and behavior (Fig. 11.1).

The traditional ILC and other ILC variants are occasionally mixed [13]. The discohesive tumor cells that make up the solid variant of ILC grow in solid nests and may exhibit pleomorphism or enhanced mitotic activity. The tumor cells of alveolar ILC are grouped in distinct clusters or aggregates of 20 cells or more, which are divided by thin fibrous septa. Tumor cells develop in bands thicker than two cells in the trabecular ILC. The tubule-lobular type of ILC has a hybrid tubular and lobular appearance. The growth pattern of pleomorphic ILC is identical to that of classic ILC, but the tumor cells exhibit increased cytological atypia and pleomorphism as well as a higher rate of mitosis [1, 12–16].

Classic ILC are of low or intermediate histological grade and the majority are characterized by the positivity of hormone receptors and lack of HER2 expression; however, HER2-positive and/or triple-negative (estrogen and progesterone receptor-negative and HER-2 negative) phenotypes have been reported, particularly in ILC variants [1, 12–18]. Consistently, more than 80% of ILCs fall into the category of luminal molecular subtypes according to gene expression profile studies [3, 19]. Her-2-enriched and basal-like lobular tumors are rare, usually of non-classic variant, and associated with a worse prognosis [20]. Similar to invasive ductal carcinoma (IDC),

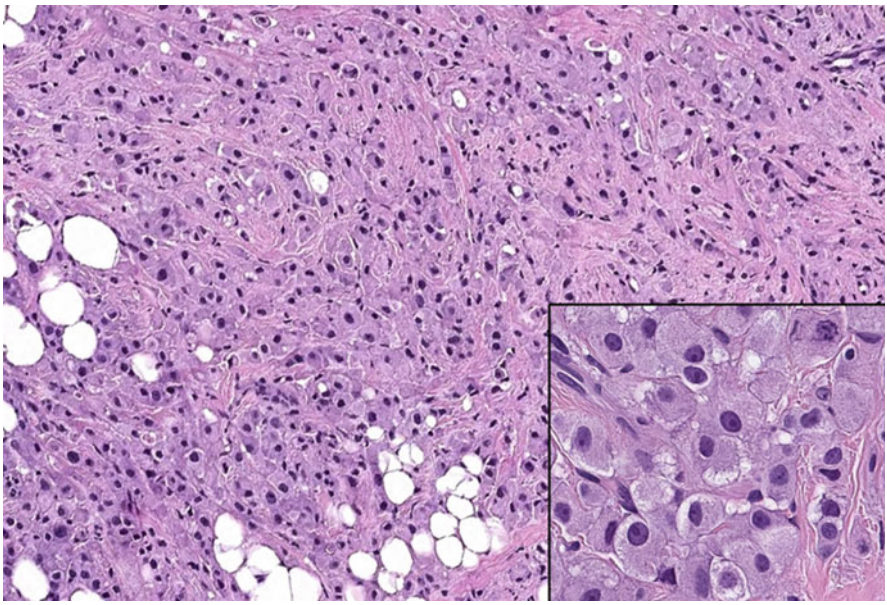


Fig. 11.1 Invasive lobular carcinoma, histiocytoid variant. These tumors are morphologically characterized by sheets/cords of cells with abundant granular cytoplasm and variably eccentric nuclei. Among the possible differential diagnoses of histiocytoid lobular carcinoma, it is worth mentioning some non-neoplastic conditions, such as reactive histiocytic infiltrates and fat necrosis. Hematoxylin and eosin, original magnification 100×; inset 400×. Note. Personal archive

tumor staging, and nodal status are important prognostic factors also in patients with ILC. Moreover, a high Ki67 proliferation index was found to be associated with a high risk of early and late recurrence [19, 20].

In addition to traditional prognostic and predictive factors, other actional biomarkers, such as tumor-infiltrating lymphocytes (TILs) and PD-L1 expression, have been recently included in the pathological characterization of IBC. PD-L1 expression in ILC has been observed both on lymphocyte and tumor cells. Overall, the level of TILs and PD-L1 reported in ILCs are lower than those observed in IDC and with different patterns, suggesting that ILC may be associated with a distinct immune microenvironment [21–24].

As mentioned above, most ILCs are currently classified as HER-2 negative. According to the American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP), the HER2 test positivity is defined by protein overexpression (score 3+) at immunohistochemistry (IHC) and/or HER2 score 2+ with gene amplification at in situ hybridization (ISH), while score 2+/ISH negative, score 1+ and score 0 were considered negative [25]. However, the introduction of novel anti-HER2 antibody-drug conjugates requires an in-depth categorization of this "HER2-negative" group, distinguishing tumors with no HER2 expression by IHC (or in less than 10% of tumor cells; score 0) from those with low HER2

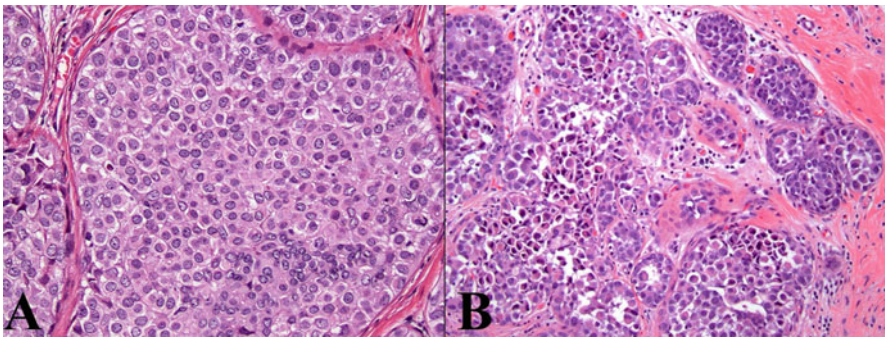


Fig. 11.2 Histological features of lobular carcinoma in situ (LCIS). (**a**) Monomorphic proliferation of polygonal discohesive cells with clear cytoplasm that distend the acini with the maintenance of the lobular architecture. (**b**) Non-invasive lesion with lobular phenotype, showing eccentric large pleomorphic nuclei, conspicuous nucleoli and large eosinophilic granular cytoplasm, consistent pleomorphic lobular carcinoma in situ. Hematoxylin and eosin, original magnification 200×. Adapted from: Guerini-Rocco and Fusco. Premalignant and preinvasive lesions of the breast. In: Breast Cancer: Innovations in Research and Management. Veronesi U, Goldhirsh A, Veronesi P, et al., editors. Springer International Publishing; 2017. p. 103–20 [33]

expression (HER2-low IBC) showing immunohistochemistry HER2 score 1+ or 2+/ISH- [26–28]. Considering ILC, fewer cases have been observed among HER2-low IBC compared to HER2-zero tumors [29, 30].

Non-invasive lobular neoplasia, including lobular carcinoma in situ (LCIS) and atypical lobular hyperplasia (ALH), are frequently seen in combination with ILC [31–34]. ALH and LCIS are considered risk indicators and non-obligate precursors of invasive breast cancer [35, 36]. The neoplastic cells of ALH/LCIS morphologically resemble those of ILC distending the acini with the maintenance of the lobular architecture. Moreover, akin to the invasive counterpart, these types of non-invasive lobular neoplasia lack E-cadherin expression, confirming the early oncogenicity of *CDH1* alterations in hereditary and non-hereditary lobular breast cancer [35–37] (Fig. 11.2).

11.3 *CDH1* Aberrations: The Hallmark of Lobular Breast Cancer

The *CDH1* gene (16q22.1) encodes for the E-cadherin protein, which is responsible for cell adhesion and suppresses cell motility and invasion [38, 39]. The rationale for the use of E-cadherin as a biomarker in ILC is related to its very biology. This protein has an extracellular domain responsible for cell-to-cell adhesion via homodimerization with other E-cadherin molecules on adjacent cells [40]. The intracellular domain interacts with the actin cytoskeleton indirectly, through a complex formed by several mediators such as α-, β-, and p120-catenins. Therefore, the presence and functionality of E-cadherin are crucial not only in maintaining

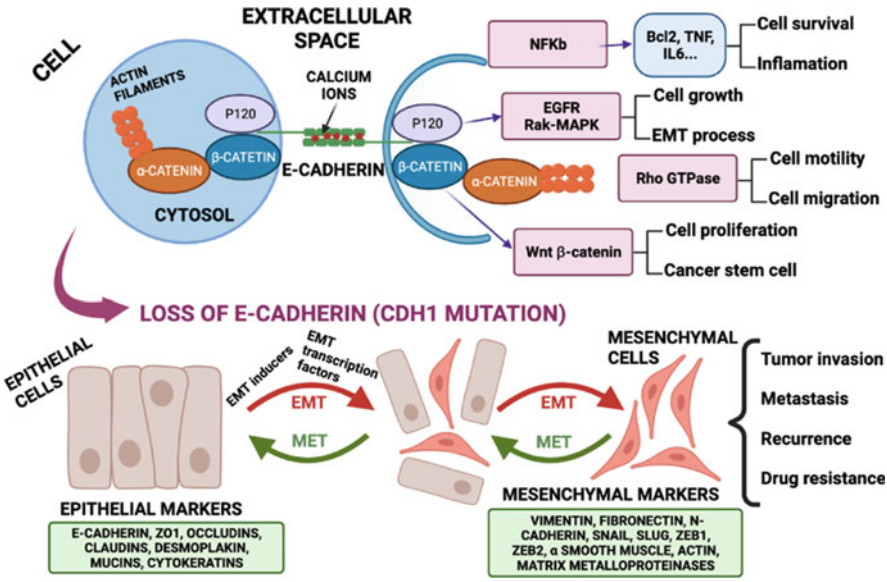


Fig. 11.3 Molecular events mediated by loss of E-cadherin in hereditary lobular carcinoma. E-cadherin is a 120 kDa glycoprotein encoded by the CDH1 gene, located on chromosome 16q22.1, and belongs to the classical Cadherin subgroup. It has an extracellular domain formed by five extracellular ~100 amino acid residue motifs, termed extracellular cadherin repeats. The calcium binding sites are located in the pockets between the repeats. This extracellular domain is mainly responsible for cell-to-cell adhesion via homodimerization with other E-cadherin molecules present on adjacent cells. E-cadherin has a single transmembrane domain that links the extracellular domain with the smaller intracellular domain. The intracellular domain interacts with the actin cytoskeleton indirectly, through a complex formed by several mediators such as α-, β-, and p120-catenins. Therefore, the presence and functionality of E-cadherin are crucial not only in maintaining cell-to-cell adhesion but through the interaction with these mediators, which plays also a role in a variety of intracellular pathways

cell-to-cell adhesion but through the interaction with these mediators, in different intracellular pathways [40, 41] (Fig. 11.3).

The loss of E-cadherin functionality caused by *CDH1* mutations results in the facilitation of epithelial-to-mesenchymal transition and tumorigenesis [42]. This molecular aberration is directly reflected by the non-to-poorly cohesive morphological appearance of lobular carcinoma cells and by the loss of immunohistochemical expression of E-cadherin and cytoplasmic expression of p120-catenins [43]. However, up to 15% of ILC may show E-cadherin expression and abnormal E-cadherin immunoreactivity has been seen in other breast cancer subtypes, including total absence or diminished membrane staining, and punctate or cytoplasmic expression [44, 45] (Fig. 11.4).

In the TCGA series, *CDH1* genomic aberrations have been detected in nearly 12% of all breast cancers including truncating, missense and splice-site mutations, copy number, and structural variants. Somatic *CDH1* mutations have been reported

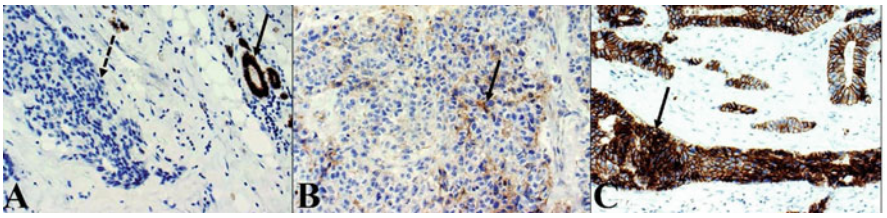


Fig. 11.4 Spectrum of E-cadherin immunoreactivity in breast cancer. Representative micrographs of (**a**) lobular carcinoma showing loss of E-cadherin immunohistochemical expression (dashed arrow) and adjacent normal terminal duct-lobular units with strong membranous E-cadherin staining (full arrow); invasive breast cancers of no special type showing partial loss (**b**) and strong (**c**) membranous immunoreactivity for E-cadherin. E-cadherin immunohistochemistry, original magnification 200×. Adapted from: Corso G, Figueiredo J, De Angelis SP, et al. E-cadherin deregulation in breast cancer. J Cell Mol Med 2020;24:5930–6 [50]

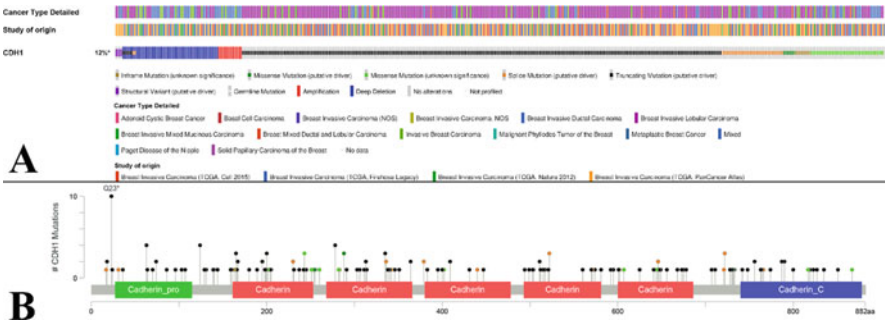


Fig. 11.5 Distribution of *CDH1* mutations in breast cancer. (**a**) Oncoprint visualization of the *CDH1* mutations across different histological subtypes of breast cancer. (**b**) Lollipop plot presenting frequencies and types of *CDH1* mutations. TGCA Combined Study (3835 samples) from https://www.cbioportal.org/, accessed 20th July 2022)

in 50–80% of lobular breast cancer [2, 3, 6] (Fig. 11.5). These mutations mostly co-occur with heterozygous loss of 16q and they are frequently associated with downregulation of CDH1 transcript and protein levels [46]. Interestingly, the complete loss of CDH1 expression alone is not sufficient for invasive carcinoma development, as demonstrated in transgenic animal models. Indeed, other genetic alterations, such as Smad4 and p53, are required to promote invasiveness and metastasis [47–49]. Besides alterations affecting the *CDH1* gene, epigenetic modifications and upregulation of transcriptional inhibitors have also been described as mechanisms of E-cadherin inactivation [50]. An important and frequent epigenetic modification is hypermethylation of the CDH1 promoter. This alteration has been studied in hereditary and non-hereditary lobular breast cancers, which suggests epigenetic silencing as an alternative CDH1 downregulation mechanism. *CDH1* DNA hypermethylation has been demonstrated to be inversely proportional to E-cadherin levels in tumor cells [51].

Interestingly, it has been observed that *CDH1* promoter hypermethylation is associated with reduced HR expression, increased disease progression, a higher metastatic rate, and a more aggressive clinical course overall. It is more frequent in patients presenting with sentinel lymph node metastases at diagnosis and is correlated with disease progression to distant metastases [52, 53]. This has led to the proposal of *CDH1* hypermethylation as a prognostic biomarker to predict poorer outcomes [54]. Another mechanism of E-cadherin inactivation is represented by the overexpression of its transcriptional inhibitors, namely Snail, SLUG, zinc finger E-box-binding (ZEB1 and 2), and TWIST transcription factors [55]. Among these molecules, the one with the highest affinity for the *CDH1* promoter is Snail, which acts by recruiting the mSin3A/Histone Deacetylase1 and 2 (HDAC1/2). Subsequent deacetylation of histones H3 and H4 results in silencing of the gene, thus effectively inhibiting E-cadherin synthesis [56, 57]. ZEB1 and ZEB2 behave similarly to Snail in suppressing *CDH1* transcription, but their mechanisms of action appear to be independent. Thus, it has been hypothesized that at least two transcriptional downregulation complexes of E-cadherin do exist, but whether they participate in tumorigenesis within the same cell remains to be established [58]. High levels of ZEB1 have been found in aggressive BCs and associated with advanced-stage and lymph node metastases. Therefore, ZEB1 has been proposed as an additional prognostic biomarker in breast cancers, in particular in lobular breast cancer [41, 50, 59–61].

E-cadherin and many RTKs tend to co-localize at the basolateral portion of the cell membrane. In particular, the complex formed by the E-cadherin intracellular domain and EGFR has been extensively studied to be involved in adhesion-dependent bidirectional crosstalk. On one hand, cell-to-cell adhesion via E-cadherin inhibits the EGFR signaling pathway, including downstream mediators such as MAPK/ERK with downregulation of cell cycle progression and cellular proliferation [62]. Conversely, it has been demonstrated that cell adhesion transiently activates the EGFR/MAPK signaling cascade, and has a role in tissue growth [63]. Moreover, the upregulation of several RTKs pathways is known to inhibit E-cadherin-dependent cell-to-cell adhesion and promote epithelial-to-mesenchymal transition (EMT), suggesting that E-cadherin plays a role in tumorigenesis even when not directly affected by inactivating mutations [64]. E-cadherin is also known to form a complex with β-catenin. The E-cadherin/ β-catenin complex is crucial in maintaining not only cell-to-cell adhesion but also tissue's architectural homeostasis. Beta-catenin is well known for being a central component of the WNT signal transduction pathway. It has been demonstrated that when catenin is bound by E-cadherin, the result is the promotion of tissue stasis by inhibition of cell proliferation and architectural stabilization. The disruption of the cadherin-catenin complex causes an increase of cytoplasmic un-bound β-catenin. This alters the WNT signaling pathway shifting the balance toward cell growth and proliferation. This effect has been demonstrated to be unrelated to E-cadherin adhesive properties and to be entirely dependent on its β-catenin binding region. In addition, β-catenin has an inhibitory effect on PTEN, a well-known tumor suppressor gene, further promoting uncontrolled cell proliferation [65, 66]. Another signaling pathway influenced by the

interaction between E-cadherin and catenins at the cell membrane is that of the Rho GTPases. The Rho GTPases are a family of proteins involved in the interaction of E-cadherin with the cytoskeleton, a process influenced also by p120-catenin. They promote and regulate the organization of the cytoskeletal network during the formation of adherens junctions. The two Rho GTPase subfamilies most known for being influenced by E-cadherin are Rac and Rho. In normal conditions, E-cadherin activates Rac1 and inhibits Rho through the interaction of p120, increasing cell adhesion and cellular structural stability. Loss of E-cadherin causes an increase in unbound p120, which in turn creates an inversion of this balance. This not only promotes loss of cell-to-cell adhesion by disruption of the adherens junctions but also enhances cellular motility and migration due to rearrangement of the cytoskeletal network. Therefore, the Rho GTPase family has an important role in the process of EMT mediated by E-cadherin loss [67, 68]. Moreover, increased levels of p120 upregulate the NF-kB pathway, which contributes to tumorigenesis by promoting inflammation, cell proliferation, and apoptosis escape [69]. During EMT, when cells have detached from their tissue of origin they start to migrate within the extracellular matrix. E-cadherin loss has been demonstrated to enhance cellular motility in this new environment by upregulation of secretion and activity of metalloproteinases (MMP) [70]. These molecules play a role in matrix digestion and remodeling and, when their activity is increased, tumor cell migration is facilitated. In addition, MMPs have been shown to inactivate E-cadherin by cleavage of its extracellular domain, further demonstrating the close interplay of these two effectors in tumor spread [71]. Besides the loss of cell-to-cell adhesion and EMT, E-cadherin loss also increases the resistance of cells to apoptotic stimuli. This effect is mediated by the inverse relationship between E-cadherin expression and the Notch pathway. Reduction in E-cadherin levels is correlated with upregulation of this pathway, leading to an increase in intracellular levels of Bcl-2. The Bcl-2 family of proteins is known to be involved in the regulation of programmed cell death. Specifically, they have an anti-apoptotic role, thus their upregulation following E-cadherin loss promotes tumor resistance to apoptotic stimuli and improves the survival of neoplastic cells [72]. The interplay between E-cadherin and a plethora of intracellular signaling pathways demonstrates how the role of this molecule in tumorigenesis goes well beyond the loss of cell-to-cell adhesion. This also highlighted the need for detailed characterization and reporting of *CDH1* variants identified, especially at the germline level.

11.4 The Genomic Landscape of Lobular Breast Cancer

During the last decades, broad genomic profiling with high-throughput next-generation sequencing technologies has shown that breast cancers are highly heterogeneous at the molecular level harboring few recurrent genomic aberrations and potentially actionable drivers [2, 4, 5, 73–77]. Overall, *PIK3CA* and *TP53* are the most frequently mutated genes with different mutation rates based on breast cancer subtype. Nearly 40% of estrogen receptor-positive/luminal breast cancer harbor

somatic driver mutations in the *PIK3CA* gene. *TP53* mutations can be detected in 20–30% of luminal tumors but nearly 85% of basal-like/triple-negative breast cancers. Indeed, these triple-negative tumors show also high genomic instability and DNA repair gene aberrations, including *BRCA1/2* alterations [75, 77]. ILC represents a special breast cancer type also at the genomic level. As mentioned above, ILC is characterized by a higher rate of *CDH1* mutations as compared to IDC (63% versus 2% in the TCGA study). Other recurrently mutated genes (reported rate > 2%) in ILC included: *PIK3CA, TBX3, RUNX1, FOXA1, ERBB2, ERBB3, PTEN, MAP3K1, AKT1, ARID1A,* and *TP53*. Besides *CDH1* heterozygous deletion (16q loss) detected in more than 90% of the cases, other recurrent copy number variations involve gain of *CCND1, FGFR1,* and *MYC* genes. Although amplification of the HER2 gene is not frequently seen in ILC, somatic mutations of *ERBB2* have been reported in 2%–15% of cases [2–6, 8, 9, 78]. Overall, as compared to estrogen receptor-positive luminal breast cancer, invasive lobular carcinoma is enriched for *CDH1* mutations and loss, mutation of *TBX3* and *FOXA1*, mutation, and loss of PTEN with activation of AKT pathway but low mutation rate of *GATA-3* [3] (Fig. 11.6).

Triple-negative (hormone receptors-negative and HER2-negative) ILC is a rare disease accounting for nearly 1% of triple-negative breast cancers and it has a poor prognosis. Although no significant differences in gene mutation frequency have been found compared to hormone receptor-positive/her2-negative cases, enrichment for alterations in ErbB and androgen receptor signaling pathways were observed in triple-negative ILC. Moreover, these tumors show a genomic profile distinct from triple-negative IDCs, including higher frequencies of *CDH1*, *ERBB2*, *PI3KCA*, and *FOXA1* mutations [8, 79, 80].

Considering primary and metastatic ILC, similar repertoires of genomic alterations have been described. However, in the metastatic setting higher frequencies of *TP53*, *ESR1*, *NF1,* and *ERRB2* alterations have been reported. Indeed, these genomic alterations may represent mechanisms of endocrine therapy resistance. Moreover, a higher tumor mutational burden has been observed in metastatic ILC as compared to primary tumors [81].

11.5 Conclusion

Lobular breast cancers display peculiar characteristics including morphologic, phenotypic, and transcriptomic features, genomic aberrations, immune microenvironment composition, and clinical behavior. Given the rarity of and maybe low awareness about hereditary CDH1-related ILC, few studies have been specifically focused on this entity and, so far, similar characteristics have been reported. Dedicated investigations are warranted to elucidate the molecular profiles of ILC that arise in women harboring *CDH1* germline mutations. Indeed, there are numerous questions to be uncovered in the molecular mechanisms driving tumorigenesis and disease progression. A focused characterization of the molecular profile of hereditary CDH1-related ILC may enhance our understanding of these tumors and ultimately

Fig. 11.6 Recurrent genomic alterations in *CDH1*-mutated invasive lobular carcinoma. Oncoprint visualization of the most frequently mutated genes in lobular breast carcinomas harboring somatic *CDH1* mutations. TGCA Firehose Legacy series (99 samples) from https://www.cbioportal.org/, accessed 20th July 2022

might aid in establishing effective prevention, screening, and tailored treatment strategies for women carrying *CDH1* germline mutations.

References

1. Breast Tumours (2019) WHO Classification of Tumours. International Agency for Research on Cancer
2. Pereira B, Chin SF, Rueda OM et al (2016) The somatic mutation profiles of 2,433 breast cancers refines their genomic and transcriptomic landscapes. Nat Commun 7:11479
3. Ciriello G, Gatza ML, Beck AH et al (2015) Comprehensive molecular portraits of invasive lobular breast cancer. Cell 163:506–519
4. Grote I, Bartels S, Christgen H et al (2022) ERBB2 mutation is associated with sustained tumor cell proliferation after short-term preoperative endocrine therapy in early lobular breast cancer. Mod Pathol
5. WHO Classification of Tumours Editorial Board (2019) WHO classification of breast tumours: WHO classification of tumours, vol 2. World Health Organization
6. Ross JS, Wang K, Sheehan CE et al (2013) Relapsed classic E-cadherin (CDH1)-mutated invasive lobular breast cancer shows a high frequency of HER2 (ERBB2) gene mutations. Clin Cancer Res 19:2668–2676
7. Corso G, Montagna G, Figueiredo J et al (2020) Hereditary gastric and breast cancer syndromes related to CDH1 germline mutation: a multidisciplinary clinical review. Cancers (Basel) 12
8. Desmedt C, Zoppoli G, Gundem G et al (2016) Genomic characterization of primary invasive lobular breast cancer. J Clin Oncol 34:1872–1881
9. Guiu S, Wolfer A, Jacot W et al (2014) Invasive lobular breast cancer and its variants: how special are they for systemic therapy decisions? Crit Rev Oncol Hematol 92:235–257
10. Chen Z, Yang J, Li S et al (2017) Invasive lobular carcinoma of the breast: a special histological type compared with invasive ductal carcinoma. PLoS One 12:e0182397
11. McCart Reed AE, Kutasovic JR, Lakhani SR et al (2015) Invasive lobular carcinoma of the breast: morphology, biomarkers and 'omics. Breast Cancer Res 17:12
12. Pagni F, Guerini-Rocco E, Schultheis AM et al (2019) Targeting immune-related biological processes in solid tumors: we do need biomarkers. Int J Mol Sci 20
13. Arias-Stella JA, Alvarado-Cabrero I, Pareja F (2018) Special types of invasive breast carcinoma. Practical Atlas of breast pathology. Springer, pp 263–292
14. Walker RA, Hanby A, Pinder SE et al (2012) Current issues in diagnostic breast pathology. J Clin Pathol 65:771–785
15. De Schepper M, Vincent-Salomon A, Christgen M et al (2022) Results of a worldwide survey on the currently used histopathological diagnostic criteria for invasive lobular breast cancer. Mod Pathol
16. Christgen M, Cserni G, Floris G et al (2021) Lobular breast cancer: histomorphology and different concepts of a special spectrum of tumors. Cancers (Basel) 13
17. Fusco N, Sajjadi E, Venetis K et al (2022) Low-risk triple-negative breast cancers: clinicopathological and molecular features. Crit Rev Oncol Hematol 103643
18. Venetis K, Crimini E, Sajjadi E, et al. HER2 low, ultra-low, and novel complementary biomarkers: expanding the spectrum of HER2 positivity in breast cancer. Front Mol Biosci 2022:fmolb.2022.834651
19. Conforti F, Pala L, Pagan E et al (2019) Endocrine-responsive lobular carcinoma of the breast: features associated with risk of late distant recurrence. Breast Cancer Res 21:153
20. Iorfida M, Maiorano E, Orvieto E et al (2012) Invasive lobular breast cancer: subtypes and outcome. Breast Cancer Res Treat 133:713–723

21. Thompson ED, Taube JM, Asch-Kendrick RJ et al (2017) PD-L1 expression and the immune microenvironment in primary invasive lobular carcinomas of the breast. Mod Pathol 30:1551–1560
22. Dill EA, Gru AA, Atkins KA et al (2017) PD-L1 expression and intratumoral heterogeneity across breast cancer subtypes and stages: an assessment of 245 primary and 40 metastatic tumors. Am J Surg Pathol 41:334–342
23. Desmedt C, Salgado R, Fornili M et al (2018) Immune infiltration in invasive lobular breast cancer. J Natl Cancer Inst 110:768–776
24. Sobral-Leite M, Van de Vijver K, Michaut M et al (2018) Assessment of PD-L1 expression across breast cancer molecular subtypes, in relation to mutation rate, BRCA1-like status, tumor-infiltrating immune cells and survival. Onco Targets Ther 7:e1509820
25. Wolff AC, Hammond MEH, Allison KH et al (2018) Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. J Clin Oncol 36:2105–2122
26. Tarantino P, Hamilton E, Tolaney SM et al (2020) HER2-low breast cancer: pathological and clinical landscape. J Clin Oncol 38:1951–1962
27. Venetis K, Crimini E, Sajjadi E et al (2022) HER2 low, ultra-low, and novel complementary biomarkers: expanding the spectrum of HER2 positivity in breast cancer. Front Mol Biosci 9:834651
28. Sajjadi E, Venetis K, Ivanova M et al (2022) Improving HER2 testing reproducibility in HER2-low breast cancer. Cancer Drug Resist 5 (Accepted)
29. Mutai R, Barkan T, Moore A et al (2021) Prognostic impact of HER2-low expression in hormone receptor positive early breast cancer. Breast 60:62–69
30. Tarantino P, Jin Q, Tayob N et al (2022) Prognostic and biologic significance of ERBB2-low expression in early-stage breast cancer. JAMA Oncol
31. Lopez-Garcia MA, Geyer FC, Lacroix-Triki M et al (2010) Breast cancer precursors revisited: molecular features and progression pathways. Histopathology 57:171–192
32. Sciarra A, Lopez G, Corti C et al (2019) Columnar cell lesion and apocrine hyperplasia of the breast: is there a common origin? The role of prolactin-induced protein. Appl Immunohistochem Mol Morphol 27:508–514
33. Guerini-Rocco E, Fusco N (2017) Premalignant and preinvasive lesions of the breast. In: Veronesi U, Goldhirsh A, Veronesi P et al (eds) Breast cancer: innovations in research and management. Springer International Publishing, pp 103–120
34. Sciarra A, Lopez G, Corti C, et al (2017) Columnar cell lesion and apocrine hyperplasia of the breast: is there a common origin? The role of prolactin-induced protein. Appl Immunohistochem Mol Morphol
35. Sakr RA, Schizas M, Carniello JV et al (2016) Targeted capture massively parallel sequencing analysis of LCIS and invasive lobular cancer: repertoire of somatic genetic alterations and clonal relationships. Mol Oncol 10:360–370
36. Lee JY, Schizas M, Geyer FC et al (2019) Lobular carcinomas in situ display intralesion genetic heterogeneity and clonal evolution in the progression to invasive lobular carcinoma. Clin Cancer Res 25:674–686
37. Girardi A, Magnoni F, Vicini E et al (2022) CDH1 germline mutations in families with hereditary lobular breast cancer. Eur J Cancer Prev 31:274–278
38. Massari G, Magnoni F, Favia G et al (2021) Frequency of CDH1 germline mutations in non-gastric cancers. Cancers (Basel) 13
39. Paço A, Leitão-Castro J, Freitas R (2021) Epigenetic regulation of CDH1 is altered after HOXB7-silencing in MDA-MB-468 triple-negative breast cancer cells. Genes (Basel) 12
40. Halbleib JM, Nelson WJ (2006) Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. Genes Dev 20:3199–3214
41. Paredes J, Figueiredo J, Albergaria A et al (2012) Epithelial E- and P-cadherins: role and clinical significance in cancer. Biochim Biophys Acta 1826:297–311

42. Corso G (2022) Pleiotropic cancer manifestations of germline CDH1 mutations: risks and management. J Surg Oncol 125:1326–1331
43. Rätze MAK, Koorman T, Sijnesael T et al (2022) Loss of E-cadherin leads to Id2-dependent inhibition of cell cycle progression in metastatic lobular breast cancer. Oncogene 41:2932–2944
44. Arps DP, Healy P, Zhao L et al (2013) Invasive ductal carcinoma with lobular features: a comparison study to invasive ductal and invasive lobular carcinomas of the breast. Breast Cancer Res Treat 138:719–726
45. Christgen M, Kandt LD, Antonopoulos W et al (2022) Inter-observer agreement for the histological diagnosis of invasive lobular breast carcinoma. J Pathol Clin Res 8:191–205
46. Cerami E, Gao J, Dogrusoz U et al (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2:401–404
47. Luo W, Fedda F, Lynch P et al (2018) CDH1 gene and hereditary diffuse gastric cancer syndrome: molecular and histological alterations and implications for diagnosis and treatment. Front Pharmacol 9:1421
48. Park JW, Jang SH, Park DM et al (2014) Cooperativity of E-cadherin and Smad4 loss to promote diffuse-type gastric adenocarcinoma and metastasis. Mol Cancer Res 12:1088–1099
49. Park JW, Kim M-S, Voon DC et al (2018) Multi-omics analysis identifies pathways and genes involved in diffuse-type gastric carcinogenesis induced by E-cadherin, p53, and Smad4 loss in mice. Mol Carcinog 57:947–954
50. Corso G, Figueiredo J, De Angelis SP et al (2020) E-cadherin deregulation in breast cancer. J Cell Mol Med 24:5930–5936
51. Shargh SA, Sakizli M, Khalaj V et al (2014) Downregulation of E-cadherin expression in breast cancer by promoter hypermethylation and its relation with progression and prognosis of tumor. Med Oncol 31:250
52. Shinozaki M, Hoon DS, Giuliano AE et al (2005) Distinct hypermethylation profile of primary breast cancer is associated with sentinel lymph node metastasis. Clin Cancer Res 11:2156–2162
53. Sebova K, Zmetakova I, Bella V et al (2011) RASSF1A and CDH1 hypermethylation as potential epimarkers in breast cancer. Cancer Biomark 10:13–26
54. Liu J, Sun X, Qin S et al (2016) CDH1 promoter methylation correlates with decreased gene expression and poor prognosis in patients with breast cancer. Oncol Lett 11:2635–2643
55. Schmalhofer O, Brabletz S, Brabletz T (2009) E-cadherin, beta-catenin, and ZEB1 in malignant progression of cancer. Cancer Metastasis Rev 28:151–166
56. Bolós V, Peinado H, Pérez-Moreno MA et al (2003) The transcription factor Slug represses E-cadherin expression and induces epithelial to mesenchymal transitions: a comparison with snail and E47 repressors. J Cell Sci 116:499–511
57. Peinado H, Ballestar E, Esteller M et al (2004) Snail mediates E-cadherin repression by the recruitment of the Sin3A/histone deacetylase 1 (HDAC1)/HDAC2 complex. Mol Cell Biol 24: 306–319
58. Comijn J, Berx G, Vermassen P et al (2001) The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion. Mol Cell 7:1267–1278
59. Figueiredo J, Melo S, Carneiro P et al (2019) Clinical spectrum and pleiotropic nature of CDH1 germline mutations. J Med Genet 56:199–208
60. Li M, Rao X, Cui Y et al (2021) The keratin 17/YAP/IL6 axis contributes to E-cadherin loss and aggressiveness of diffuse gastric cancer. Oncogene
61. Xiang S, Liu YM, Chen X et al (2015) ZEB1 expression is correlated with tumor metastasis and reduced prognosis of breast carcinoma in Asian patients. Cancer Investig 33:225–231
62. Qian X, Karpova T, Sheppard AM et al (2004) E-cadherin-mediated adhesion inhibits ligand-dependent activation of diverse receptor tyrosine kinases. EMBO J 23:1739–1748
63. Pece S, Gutkind JS (2000) Signaling from E-cadherins to the MAPK pathway by the recruitment and activation of epidermal growth factor receptors upon cell-cell contact formation. J Biol Chem 275:41227–41233
64. Thiery JP (2002) Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2: 442–454

65. Gottardi CJ, Wong E, Gumbiner BM (2001) E-cadherin suppresses cellular transformation by inhibiting beta-catenin signaling in an adhesion-independent manner. J Cell Biol 153:1049–1060
66. Nelson WJ, Nusse R (2004) Convergence of Wnt, beta-catenin, and cadherin pathways. Science 303:1483–1487
67. Deplazes J, Fuchs M, Rauser S et al (2009) Rac1 and Rho contribute to the migratory and invasive phenotype associated with somatic E-cadherin mutation. Hum Mol Genet 18:3632–3644
68. Bruner HC, Derksen PWB (2018) Loss of E-cadherin-dependent cell-cell adhesion and the development and progression of cancer, vol 10. Cold Spring Harb Perspect Biol
69. Vlahopoulos SA, Cen O, Hengen N et al (2015) Dynamic aberrant NF-κB spurs tumorigenesis: a new model encompassing the microenvironment. Cytokine Growth Factor Rev 26:389–403
70. Munshi HG, Ghosh S, Mukhopadhyay S et al (2002) Proteinase suppression by E-cadherin-mediated cell-cell attachment in premalignant oral keratinocytes. J Biol Chem 277:38159–38167
71. Davies G, Jiang WG, Mason MD (2001) Matrilysin mediates extracellular cleavage of E-cadherin from prostate cancer cells: a key mechanism in hepatocyte growth factor/scatter factor-induced cell-cell dissociation and in vitro invasion. Clin Cancer Res 7:3289–3297
72. Ferreira AC, Suriano G, Mendes N et al (2012) E-cadherin impairment increases cell survival through notch-dependent upregulation of Bcl-2. Hum Mol Genet 21:334–343
73. Christinat A, Pagani O (2013) Practical aspects of genetic counseling in breast cancer: lights and shadows. Breast 22:375–382
74. Stephens PJ, Tarpey PS, Davies H et al (2012) The landscape of cancer genes and mutational processes in breast cancer. Nature 486:400–404
75. The Cancer Genome Atlas Network (2012) Comprehensive molecular portraits of human breast tumours. Nature 490:61–70
76. Curtis C, Shah SP, Chin SF et al (2012) The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature 486:346–352
77. Ellis MJ, Perou CM (2013) The genomic landscape of breast cancer as a therapeutic roadmap. Cancer Discov 3:27–34
78. Corso G, Veronesi P, Sacchini V et al (2018) Prognosis and outcome in CDH1-mutant lobular breast cancer. Eur J Cancer Prev 3:237–238
79. Bareche Y, Venet D, Ignatiadis M et al (2018) Unravelling triple-negative breast cancer molecular heterogeneity using an integrative multiomic analysis. Ann Oncol 29:895–902
80. Conforti F, Pala L, Pagan E et al (2021) Biological and clinical features of triple negative invasive lobular carcinomas of the breast. Clinical outcome and actionable molecular alterations. Breast 59:94–101
81. Pareja F, Ferrando L, Lee SSK et al (2020) The genomic landscape of metastatic histologic special types of invasive breast cancer. NPJ Breast Cancer 6:53

Part IV

Endoscopy and Imaging

Endoscopy: Is There Anything New?

12

Cristina Trovato

Abstract

Current guidelines recommend upper endoscopy in *CDH1* carriers prior to surgery and then annually for individuals deferring prophylactic gastrectomy. However, endoscopic detection of cancer foci in HDGC is suboptimal and imperfect for facilitating decision-making. Alternative endoscopic modalities such as chromoendoscopy, endoscopic ultrasound, and other non-white light methods have been utilized, but are of limited utility to further improve cancer detection and risk stratification in HDGC.

12.1 Text Content and Description

Current guidelines recommended upper endoscopy in CDH1 carriers prior to surgery and then annually for individuals who defer prophylactic total gastrectomy. However, when endoscopic surveillance is offered, the limitations should be discussed with the patient.

The IGCLC endoscopy surveillance protocol (Cambridge method) prescribes a careful examination in a dedicated session of at least 30 min with high-definition white light in a centre of expertise. Prior to obtaining random gastric biopsies, targeted biopsies of all suspicious lesions, in particular pale areas (considered more likely to have abnormal signet ring cells), erythema, erosion, or other gastric abnormalities, should be taken. After sampling all visible lesions, five random biopsies should then be taken from six anatomic regions (prepyloric, antrum, transitional zone, body, fundus, and cardia) [1–3].

C. Trovato (✉)
Endoscopy Division, European Institute of Oncology, IRCCS, Milan, Italy
e-mail: cristina.trovato@ieo.it

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_12

The primary goal of surveillance endoscopy is to assess for gastric mucosal changes that may signal progression of early cancer foci and exclude more infiltrative (>T1a) lesions. In addition, the results of surveillance endoscopy can provide patients with the opportunity to make more informed decisions about gastrectomy. However, endoscopic surveillance often fails to detect microscopic disease and histological evaluation of surgical specimens demonstrates cancer foci in up to 45–60% of cases with a negative endoscopic evaluation [4, 5].

Given its poor reproducibility and high false-negative rates, techniques of early gastric cancer surveillance other than the Cambridge method have been explored.

At early stage, in diffuse gastric cancer (DGC) the neoplastic cells begin infiltrating the mucosa, while preserving a normal surface epithelium. Thus, endoscopy findings can remain normal until the late stages of the disease leading to a delay in the diagnosis and a very poor prognosis. Moreover, signet-ring cell carcinoma (SRCC) foci can be sparse (less than 2% of the gastric mucosa) and each focus is very often less than 1 mm in greatest diameter [6].

A model developed by Fujita et al. estimated that for a 90% detection rate, the theoretical number of biopsies necessary is 1768 per patient, but this is not clinically feasible [7]. Moreover, the main disadvantage of taking an extensive number of biopsies is the formation of scar tissue, which can then mimic the superficial pale appearance of SRCC lesions.

To improve the diagnostic performance for early HDGC lesions, it is essential to recognize and well describe the characteristic endoscopic features of those tumours. Early-stage, superficial SRCC can be seen as non-elevated pale lesions during gastroscopy (Fig. 12.1). This was first demonstrated by Shaw et al. using chromoendoscopy with Congo red–methylene blue staining to enhance the visibility of such lesions [8]. Mi et al. reported that targeted biopsies (of typical pale lesions) can result in detection of SRCC foci in more than 40% of patients, yielding a sensitivity of 28% [9]. However, we have to consider other studies demonstrating that pale areas are very non-specific for SRCC [10–12].

More recent publications have demonstrated that pale lesions can also be found using white-light endoscopy combined with narrow-band imaging (NBI) to enhance their visibility. Multiple lesions of early HDGC in white-light imaging, narrow-band imaging (NBI), and magnifying endoscopy with NBI were showed also in a video report [13].

Van Dieren et al. reported that, in a cohort of CDH1 mutation carriers, SRCC lesions were identified by an extensive endoscopic surveillance protocol in 69% of SRCC-positive patients who underwent a gastric resection. NBI was added as standard to the guideline protocol for close inspection of the entire mucosal wall following white-light endoscopy. After targeted biopsies had been taken, six random biopsies were taken from five anatomic regions (antrum, transitional zone, body, fundus, and cardia). In this paper, the yield of targeted biopsies (11%) was much higher for identification of SRCC lesions than the yield of random biopsies (0.9%). The low number of SRCC detected through random sampling demands a critical reappraisal of random biopsy sampling in the IGCLC guideline [4].

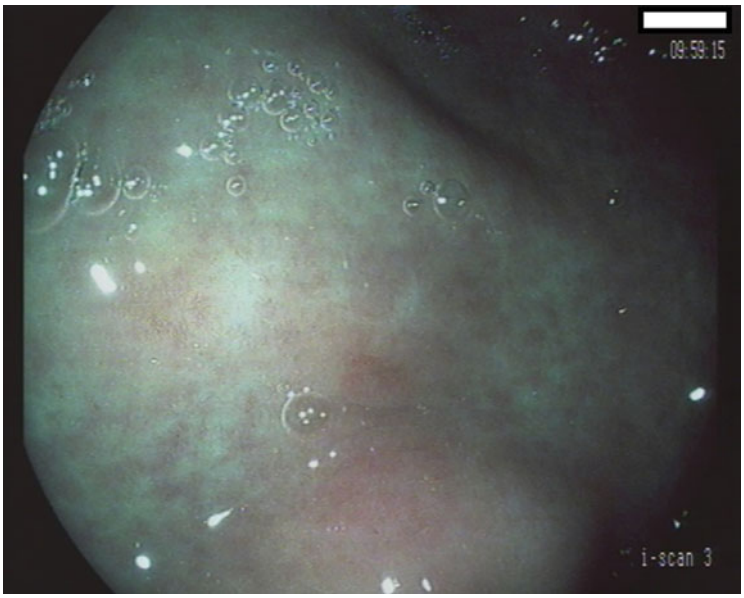


Fig. 12.1 Example of non-elevated pale lesion representing foci of superficial pT1a signet-ring cell carcinoma viewed using high-definition scope with i-Scan system (Pentax, Tokyo, Japan)

The Bethesda protocol is a systematic visualization and biopsy approach of the gastric mucosa adapted from a method previously described by Yao [14]. After a standard endoscopic examination, 22 separate anatomic sites were examined and photographed. Four non-targeted biopsies were obtained from each of the 22 sites. Abnormal findings were biopsied in addition to the systematic biopsies. On a per endoscopy basis, the false-negative rates of detection using Cambridge method and Bethesda protocol were 80% (12/15) and 37.7% (17/45), respectively ($p < 0.01$) [15].

Chromoendoscopy, which aids in identifying mucosal pale areas, was reported to improve SRCC detection rates; however, this technique is limited to detecting only larger cancer lesions. Moreover, due to concerns about dye toxicity, chromoendoscopic examination is currently not recommended as a standard of care for HDGC [1, 12, 16].

Further development of endoscopic techniques, such as electronic enhanced imaging techniques, confocal endomicroscopy, magnification and artificial intelligence, is warranted to improve the detection rate of SRCC foci.

The implementation of artificial intelligence (AI) technologies across multiple gastrointestinal (GI) endoscopic applications has the potential to transform clinical practice favourably and improve the efficiency and accuracy of current diagnostic methods.

Recently, AI-assisted convolutional neural network (CNN) computer-aided diagnosis (CAD) system, based on magnifying endoscopy with narrow-band imaging

(ME-NBI) images, was proposed and evaluated for diagnosis of early gastric cancer (EGC), but has not been studied in HDGC. This system may have great potential for future application to real clinical settings, which could facilitate ME-NBI diagnosis of EGC in practice [17].

Confocal endomicroscopy (CEM) is indicated for microscopic visualization of the mucosa during endoscopy at an approximately 1000-fold magnification and might limit the sampling error of untargeted biopsies [18]. However, in a recent phase II clinical trial, confocal endomicroscopy alone has low sensitivity for occult cancer detection in *CDH1* variant carriers, although it appeared no worse than the current recommended method and required fewer biopsies per patient [19].

Note

Parts of this chapter are based on the open access publications by Corso et al. 2020 [20] and Corso et al. 2022 [21].

References

1. van der Post RS, Vogelaar IP, Carneiro F et al (2015) Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. J Med Genet 52:361–374
2. Blair VR, McLeod M, Carneiro F et al (2020) Hereditary diffuse gastric cancer: updated clinical practice guidelines. Lancet Oncol 21(8):e386–e397
3. Treese C, Siegmund B, Daum S (2022) Hereditary diffuse gastric cancer-update based on the current consort recommendations. Curr Oncol 29(4):2454–2460
4. Van Dieren JM, Kodach LL, Hartog PD et al (2020) Gastroscopic surveillance with targeted biopsies compared with random biopsies in CDH1 mutation carriers. Endoscopy 52(10): 839–846
5. Friedman M, Adar T, Patel D et al (2021) Surveillance endoscopy in the management of hereditary diffuse gastric cancer syndrome. Clin Gastroenterol Hepatol 19(1):189–191
6. Huntsman DG, Carneiro F, Lewis FR et al (2001) Early gastric cancer in young, asymptomatic carriers of germ-line E-cadherin mutations. N Engl J Med 344(25):1904–1909
7. Fujita H, Lennerz JKM, Chung DC et al (2012) Endoscopic surveillance of patients with hereditary diffuse gastric cancer: biopsy recommendations after topographic distribution of cancer foci in a series of 10 CDH1-mutated gastrectomies. Am J Surg Pathol 36(11):1709–1717
8. Shaw D, Blair V, Framp A et al (2005) Chromoendoscopic surveillance in hereditary diffuse gastric cancer: an alternative to prophylactic gastrectomy? Gut 54:461–468
9. Mi EZ, Mi EZ, di Pietro M et al (2018) Comparative study of endoscopic surveillance in hereditary diffuse gastric cancer according to CDH1 mutation status. Gastrointest Endosc 87: 408–418
10. Jacobs MF, Dust H, Koeppe ES et al (2019) Outcomes of endoscopic surveillance in individuals with genetic predisposition to hereditary diffuse gastric cancer. Gastroenterology 157(1):87–96
11. Lim YC, Di Pietro M, O'Donovan M et al (2014) Prospective cohort study assessing outcomes of patients from families fulfilling criteria for hereditary diffuse gastric cancer undergoing endoscopic surveillance. Gastrointest Endosc 80(1):78–87
12. de Almeida Artifon EL, Marinho FRT (2018) Endoscopic screening for hereditary diffuse gastric cancer: one size does not fit all. Gastrointest Endosc 87(2):405–407
13. Namikawa K, Kawachi H, Tsugeno Y et al (2021) Detection of multiple intramucosal signet-ring cell carcinomas by white-light endoscopy and magnifying endoscopy with narrow-band

imaging in a hereditary diffuse gastric cancer patient with a *CDH1* germline mutation. VideoGIE 6(4):163–166
14. Yao K (2013) The endoscopic diagnosis of early gastric cancer. Ann Gastroenterol 26:11–22
15. Curtin BF, Gamble LA, Schueler SA et al (2021) Enhanced endoscopic detection of occult gastric cancer in carriers of pathogenic CDH1 variants. J Gastroenterol 56(2):139–146
16. Ruf B, Roggia C, Schroeder C, Mattern S et al (2022) Chromoendoscopy in combination with random biopsies for patients with pathogenic CDH1 mutations undergoing endoscopic surveillance. J Gastrointest Cancer. https://doi.org/10.1007/s12029-022-00831-1
17. Ueyama H, Kato Y, Akazawa Y et al (2021) Application of artificial intelligence using a convolutional neural network for diagnosis of early gastric cancer based on magnifying endoscopy with narrow-band imaging. J Gastroenterol Hepatol 36(2):482–489
18. Park CH, Kim H, Jo JH et al (2019) Role of probe-based confocal laser endomicroscopy-targeted biopsy in the molecular and histopathological study of gastric cancer. J Gastroenterol Hepatol 34(1):84–91
19. Schueler SA, Gamble LA, Curtin BF et al (2021) Evaluation of confocal laser endomicroscopy for detection of occult gastric carcinoma in *CDH1* variant carriers. J Gastrointest Oncol 12(2): 216–225
20. Corso G, Montagna G, Figueiredo J et al (2020) Hereditary gastric and breast cancer syndromes related to CDH1 germline mutation: a multidisciplinary clinical review. Cancers (Basel) 12(6): 1598
21. Corso G, Magnoni F, Trovato C et al (2021) Hereditary gastric cancer: a new syndrome. In: Gastric cancer: the 25-year R-evolution, pp 37–50

Endoscopic Surveillance and Pathology of Biopsies in *CDH1*, *CTNNA1*, and HDGC-Like Families

13

Jolanda M. van Dieren, Tanya M. Bisseling, Liudmila L. Kodach, and Chella R. S. van der Post

Abstract

In this chapter, the endoscopic surveillance indications, limitations, and histological findings in *CDH1*, *CTNNA1,* and HDGC-like families are discussed. Annual endoscopic surveillance following an extensive protocol in an expert center is accepted as an alternative for individuals with a pathogenic *CDH1* variant who wish to postpone surgery. Since there are a limited number of *CTNNA1* families described, the penetrance data is limited, and the indication for a prophylactic gastrectomy is unclear. Prophylactic gastrectomy can be discussed depending on the personal and family history, but annual endoscopic surveillance in an expert center is mostly preferred in carriers of a pathogenic *CTNNA1* variant. Surveillance instead of prophylactic total gastrectomy is also advised in pathogenic *CDH1* variant carriers with an unclear risk of diffuse gastric cancer, such as those families who present exclusively with breast cancer, or who do not meet HDGC genetic testing criteria. First-degree relatives and affected individuals from families with a variant of unknown significance and HDGC-like families may be considered for annual endoscopic surveillance for at least 2 years. Endoscopically, early signet ring cell lesions are subtle alterations such as pale areas and sessile lesions, and this may change regarding form, erosion, and

J. M. van Dieren
Department of Gastrointestinal Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

T. M. Bisseling
Department of Gastroenterology, Radboud University Medical Centre, Nijmegen, The Netherlands

L. L. Kodach
Department of Pathology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

C. R. S. van der Post (✉)
Department of Pathology, Radboud University Medical Centre, Nijmegen, The Netherlands
e-mail: Chella.vanderpost@radboudumc.nl

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_13

vascular pattern, when lesions invade more deeply. Corresponding histological findings in biopsies from HDGC(-like) individuals can be subdivided into three categories: (1) small intramucosal signet-ring cell carcinoma, which can be considered as indolent, very early DGC lesions—T1a; (2) small to intermediate lesions with increased atypical cells, still restricted to the mucosa of the stomach—T1+ and; (3) advanced diffuse-type gastric cancer in which tumor cells are diffusely infiltrative—DGC ≥ T2.

13.1 Endoscopic Surveillance in *CDH1* and *CTNNA1*

In carriers of a *CDH1* or *CTNNA1* pathogenic germline variant, early gastric cancer presents often as subtle lesions that are typical for this group. Both the early superficial lesions and the more advanced lesions can be very difficult to find. As such, surveillance endoscopy performed in hereditary diffuse gastric cancer (HDGC) demands specific experience. The percentage of persons with a *CDH1* pathogenic germline variant that are identified with signet ring cell carcinoma (SRCC) lesions during endoscopy shows a great variation in the literature and differs between 9 and 61% [1–7]. In most publications SRCCs are mainly found in random biopsies, although some experienced centers also report a yield of 28–43% of mutation carriers with positive targeted biopsies (Table 13.1). The large variation in the yield of targeted biopsies may depend on experience of the endoscopist with this specific group of patients and the inspection time. Most lesions are identified during the first endoscopy and less often during follow-up endoscopies [3, 7]. Because there are few patients with *CTNNA1* pathogenic germline variants, very little is known on the yield of surveillance in this group.

Annual surveillance usually starts when a person (>18 years) tested positive for a pathogenic germline variant in *CDH1* or *CTNNA1*. There is no strict upper age limit to end the surveillance, this depends on the person's fitness and co-morbidity. But in general, surveillance in those older than 70 is probably not purposeful. The use of high-definition endoscopes, the use of contrast enhancement techniques such as narrow band imaging/i-scan/optical enhancement/Fujinon intelligent color-enhancement, the experience of the endoscopist and the pathologist, and the adherence to the protocol are all likely factors to increase the detection rate. The surveillance endoscopy is preferentially performed under conscious sedation with midazolam or with propofol sedation, as discomfort and unrest is not beneficial for an optimal inspection. The stomach is flushed with water or mucolytics to remove adherent mucus and bile. The extent of inflation and deflation should be tested two to three times. Then a thorough 30-minute close inspection of the entire mucosal wall with white light and contrast enhancement techniques is done. All suspected lesions are biopsied separately and sent for histopathological investigation. Besides these targeted biopsies, also 30 random biopsies are taken (5 cardia, 5 fundus, 10 body, 5 transitional zone, 5 antrum) [8]. It is also recommended to exclude *Helicobacter*

Table 13.1 Yield of endoscopic surveillance in *CDH1* mutation carriers

First author	Journal, year	Mutation carriers	Optical technique for targeted biopsies	Percentage of persons with SRCC positive biopsies detected by targeted biopsies	Method for random biopsies	Percentage of persons with SRCC positive biopsies by random biopsies	Total percentage of persons with SRCC positive biopsies
Shaw	Gut, 2005	33	WLE + chromoendoscopy (Congo red)	32%	No random biopsies	NA	32%
Hebbard	Ann Surg Oncol, 2009	23	WLE, otherwise not specified	0%	Random biopsies, no specified protocol	9%	9%
Chen	Ann Surg Oncol, 2011	12	WLE + chromoendoscopy (methylene blue) + EUS	0%	Random biopsies (>6), no specified protocol	17%	17%
Pandalai	Surgery, 2011	10	WLE, otherwise not specified	NA	Not specified	NA	10%
Huneburg	Endosc Int Open, 2016	7	WLE + chromoendoscopy (indigo carmine)	0%	Cambridge protocol	14%	14%
Mi	Gastrointest Endosc, 2018	54	WLE + NBI + autofluorescence	28%	Cambridge protocol	50%	61%
Jacobs	Gastroenterol, 2019	20	WLE, otherwise not specified	0%	Cambridge protocol	40%	40%
Friedman	Clin Gastroenterol Hepatol, 2019	32	WLE, otherwise not specified	0%	Cambridge protocol	22%	22%
Van Dieren	Endoscopy, 2020	42	WLE + NBI	43%	Cambridge protocol	24%	50%

(continued)

Table 13.1 (continued)

First author	Journal, year	Mutation carriers	Optical technique for targeted biopsies	Percentage of persons with SRCC positive biopsies detected by targeted biopsies	Method for random biopsies	Percentage of persons with SRCC positive biopsies by random biopsies	Total percentage of persons with SRCC positive biopsies
Vos	JAMA Surgery, 2020	142	WLE + NBI	NA	Random biopsies (25), no specified protocol	NA	18%
Curtin	J Gastroenterol 2020	120	WLE, otherwise not specified	0%	Cambridge protocol (19%), Bethesda protocol (81%)	15% Cambridge, 36% Bethesda	33%
Schueler	J Gastrointest Oncol, 2021	36	WLE + confocal microscopy with fluorescein	17%	Cambridge protocol	11%	19%

This table demonstrates an overview of publications describing the yield of endoscopic surveillance in *CDH1* mutation carriers. Abbreviations: ref = reference, WLE = white light endoscopy, EUS = endoscopic ultrasonography, NBI = narrow band imaging. Cambridge protocol contains 30 random biopsies. Bethesda protocol contains 88 random biopsies

Pylori. Dependent on the *H. pylori* test (e.g., histological analysis or bacteria culture), it might be necessary to take additional biopsies.

13.2 Type of Lesions Found in Gastroscopic Surveillance in *CDH1* (Panel Fig. 13.1)

1. *Endoscopic and Histological Features of Typical Intra-mucosal Type 1 lesions (T1a)*
   Small intra-mucosal (pT1a) foci of signet-ring cells are considered to be early lesions for infiltrative diffuse gastric cancer. Shaw et al were the first to report that

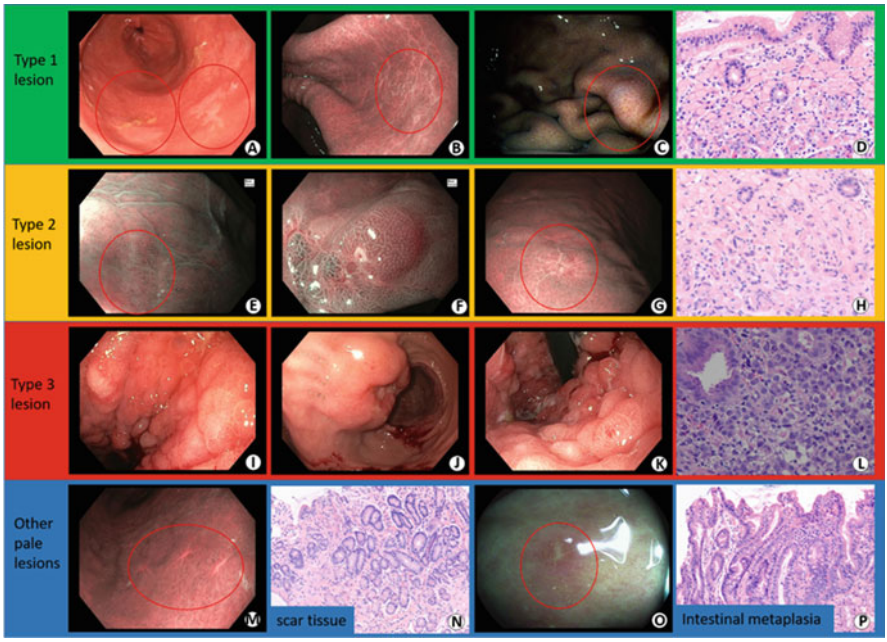


Fig. 13.1 Panel figure of endoscopic lesions and corresponding histology in biopsies of HDGC. *Type 1 lesions*: Pale SRCC lesions often have a round well-demarcated contour (**a** and **c**), but may be more spiculated (**b**). Microscopically, T1a foci are characterized by an accumulation of typical mucin-filled signet ring cells restricted to the upper layer of the mucosa (**d**). *Type 2 lesions*: T1+ lesions can show subtle depression (**e** and **g**) or elevation (**f**) and a changed chaotic, vascular pattern (**g**) or coarse pit pattern (**f**). Type 2 lesions display smaller pleiomorphic tumor cells intermingled with classic signet ring cells (**h**). *Type 3 lesions*: Endoscopically DGC can be seen as thickened folds (**i** and **k**) or as a more localized elevated tumor with ulceration (**j**). Microscopically, there is a diffuse atypical tumor mass of polymorphic atypical small cells with only some typical signet-ring cells (**l**). *Other pale lesions*: Scar tissue is characterized by a typical biopsy-forceps shape (**m**). Microscopically hardly any abnormality is seen, only subtle fibrous tissue, some irregular vessels and some irregularity of glandular architecture (**n**). Intestinal metaplasia is endoscopically visible as white plaques, often spreading over a greater area, either in antrum. or corpus or both (**o**). And microscopically typical goblet cells are seen in the glands (**p**)

small, millimetric non-elevated pale lesions found during gastroscopy represented these intramucosal clusters of signet ring cells [1]. These authors used chromoendoscopy with Congo-Red to enhance the visibility of these lesions [1]. However, since Congo-Red has been labeled as potentially carcinogenic, this technique is not used for screening anymore. More recent publications have demonstrated that the visibility can also be enhanced by adding narrow band imaging to white light endoscopy [3, 8]. The pale SRCC lesions often have a round well-demarcated contour, sometimes they are more garland-shaped or spiculated. Alternative diagnoses of non-elevated pale lesions are scar tissue/fibrosis (often induced by earlier biopsies) or intestinal metaplasia [3]. Although scar tissue induced by earlier biopsies often have a typical biopsy-forceps shape and intestinal metaplasia is often more garland-shaped than SRCC lesions, it can be very difficult to differentiate these diagnoses with the bare eye. Therefore, a biopsy is recommended if there is doubt about the endoscopic diagnosis.

The T1a foci are microscopically characterized by an accumulation of typical mucin-filled signet ring cells restricted to the lamina propria, especially the upper layer of the mucosa, without infiltration beyond the mucosa. Most of these foci are small, less than 1 mm up to several millimeters. Signet ring cells in these T1a foci are considered differentiated hypoproliferative cells. Precursor signet ring cell lesions as in-situ and pagetoid spreading signet ring cells are not regularly found in surveillance biopsies. It is unclear whether T1a foci found in HDGC patients are persistent lesions that may progress into advanced DGC or are dynamically formed and dissipated, therefore the natural course of these lesions is difficult to predict [9]. It is likely that intramucosal SRCCs can have an indolent phase, since many T1a SRCCs without deeper invasion are typically found in propylactic gastrectomies.

2. *Endoscopic and Histological Features of Atypical and Infiltrative Type 2 Lesions (T1+)*

As discussed, it is unknown if all indolent signet ring cell lesions have the ability to change to a more aggressive behavior and it is also unknown what exactly can trigger this change. Endoscopic aspects of early lesions losing their indolence have been scarcely described. However, aspects of some lesions with a changed endoscopic morphology have been described in recent publications [3, 8]. Lesions that invade more deeply than the mucosa can display erosion or ulceration, subtle depression or elevation of the lesion, a changed, chaotic vascular pattern, and also a coarse pit pattern can be seen. As mentioned, data on the lesions that are losing their indolence are scarce, but the described features should probably be regarded as endoscopic alarm symptoms. The endoscopic features should also be linked to the histopathological aspect of the lesion.

Whereas the indolent T1a (type 1) lesions display a monomorphic clustering of signet ring cells in the upper half of the lamina propria, the more invasive T1+ (type 2) lesions are characterized by an increased amount of smaller pleiomorphic tumor cells with enlarged irregular nuclei without or a minority classic signet ring cells. These cells have a tendency to spread deeper in the lamina propria and may already invade the muscularis mucosa. Also some degree of stromal reaction may

be seen. The progressive accumulation of poorly differentiated cells, as seen in pT1+ tumors, suggests that gradual changes in early-stage lesions may drive their transition into advanced DGC over time. In line with this, advanced DGC tumors in gastrectomy specimens frequently contain signet ring cells near the lumen, which may indicate the former existence of an early lesion. The type 2, T1+ lesions are rarely seen, possibly because this intermediate stage between T1a and advanced stage is a short-lived stage. Alternatively, it can be hypothesized that the development of type 1 to type 2/3 lesions represents independent events. In this case, tumor cells either form indolent non-persistent T1a lesions made up of classic signet ring cells or form from the start atypical lesions that have the ability to progress into advanced cancer [9].

3. *Endoscopic and Histological Features of Truly Advanced Lesions: Diffuse Gastric Cancer, Type 3 Lesions (≥T2)*
   The aspect of advanced diffuse gastric cancer is not uniform. Sometimes a focal tumor (mass) can be seen. Ulceration can also be present. However, more often, diffuse gastric cancer presents not as a focal circumscribed mass, but as a diffuse abnormal aspect of the stomach with thickened rigid gastric folds (linitis plastica) which makes the stomach difficult to insufflate with air. The fact that a clear circumscript lesion is often lacking and that the appearance of the thickened folds can also resemble gastritis can make it a very difficult endoscopic diagnosis. Especially since it is also known that biopsies from these thickened folds can often be false-negative as tumor cells mostly grow underneath the mucosa.

   Microscopically biopsies of advanced DGC are characterized by a mixture of diffusely growing atypical pleiomorphic cells with or without classic signet ring cells. There may be some degree of stromal reaction. Progression toward advanced DGC is accompanied by an increased abundance of poorly differentiated cells with proliferative capacity. Histologically it is difficult or even impossible to discriminate type 2 from type 3 lesions, since biopsies are restricted to the mucosa with only some amount of submucosal tissue. An indication for widely spreading DGC is when the entire gastric biopsies are filled with a diffusely growing atypical tumor proliferation with hardly any pre-existent mucosa.

13.3 Considerations on the Goals of Surveillance Endoscopy

Gastroscopic surveillance has its limitations as both advanced diffuse gastric cancer and superficial signet ring cell lesions can be very difficult to detect. Therefore, prophylactic gastrectomy should be offered to all (healthy) carriers of a pathogenic *CDH1* germline variant with a positive history of DGC in the family [8]. At least a single endoscopy is advised in all patients who consider or have already decided that they want to undergo a prophylactic gastrectomy. In these patients the purpose of the endoscopy is mainly to exclude invasive gastric cancer (≥T2), as this warrants adequate staging imaging and, in case of no further dissemination, neo-adjuvant systemic treatment [10]. Also other abnormalities or disease entities should be

excluded or treated such as Barrett's metaplasia in the distal esophagus and *Helicobacter pylori* infection. Both conditions might influence treatment. In case of a Barrett's esophagus, the surgery (the level of the anastomosis) might be adapted. Alternatively, an endoscopic resection of the Barrett segment should be considered. In case of a proven *Helicobacter pylori* infection in a patient aiming for endoscopic surveillance, the *Helicobacter pylori* should be eradicated, since it is an additional WHO-classified risk factor for the development of gastric cancer. Also, the stomach mucosa is easier to inspect endoscopically if there is no significant inflammation.

There can be multiple reasons for carriers to postpone surgery and choose for endoscopic surveillance instead. The fact that there is also a fair chance that life-threatening gastric cancer will never occur, individual co-morbidity, age, and the enormous impact of a prophylactic gastrectomy on the quality of life are possible reasons to choose for surveillance instead of a prophylactic gastrectomy. Also, families with unknown cancer risks may prefer surveillance, e.g., families without gastric cancer or with exclusively breast cancer (defined as hereditary lobular breast cancer—HLBC) and variants of unknown significance. As such, the risk of life-threatening gastric cancer is probably lower in families that do not fulfill HDGC criteria, such as families identified with multigene panel testing, HLBC families, or families with *CDH1* variants of unknown significance [4, 11]. If someone chooses to undergo surveillance, it is important that they are aware of the limitations of surveillance. These limitations should be extensively discussed with those who consider to undergo surveillance instead of a prophylactic total gastrectomy. They must be informed that surveillance can delay identification and treatment of gastric cancer. Also, surveillance should only be performed in expert centers where both endoscopists and pathologists have ample experience with this specific group of patients [8].

The overall goal of surveillance endoscopy is to determine if surgery can be postponed safely. Surveillance is probably not purposeful in those who are not able or willing to undergo treatment, especially total gastrectomy. Patients who want to postpone surgery often want to decide about the timing of surgery depending on the results of the gastroscopy. Some prefer to continue annual surveillance until a certain timepoint in their life where they feel more prepared for the impactful surgery, for example after finishing their education, after starting up their career or after birth of their children. Others will also choose for continuing surveillance despite the discovery of an early T1 lesion, although gastrectomy is formally advised, but they opt to wait until (significant) T1(+) lesions are found. Intensifying the surveillance interval to biannually is then recommended to carefully re-inspect the area of the detected lesion and possible progression towards a type 2 lesion. The surveillance is then aimed on finding increased atypia either endoscopically or histologically, implying a $\geq$ T1a + lesion. These findings warrant a gastrectomy.

The goal of gastric surveillance should not be to find every single SRCC lesion. There is a very high a priori chance of having SRCC lesions as a *CDH1* mutation carrier. A literature study of histopathological examination after total gastrectomy in 174 asymptomatic *CDH1* mutation carriers showed that 95% of the gastrectomy specimens contained T1a signet ring cell lesions when a total embedding protocol is

used [12]. Yet, only a small proportion of the T1a lesions are found during endoscopic surveillance, even in experienced centers. In a publication with one of the highest reported yields of endoscopic SRCC detection by targeted biopsies, it was calculated that at least 94% of the superficial T1a lesions were not detected during endoscopy when a comparison was made with the number of lesions detected by pathological examination of the resection specimen [3].

Furthermore, the chance for *CDH1* mutation carriers of developing life-threatening, metastatic gastric cancer is now estimated at 42% for men and 33% for women [11]. These data confirm the fact that the superficial T1a lesions can display a very indolent behavior. Altogether, the high a priori chance of having superficial T1a lesions, the low chance of finding them even in state-of-the-art endoscopic surveillance and their indolent behavior raise questions on the relevance of finding these in surveillance endoscopy. Therefore, the detection of superficial T1a lesions without atypical features must not lead to a prompt advice to undergo surgery in a surveillance setting, especially in older-aged mutation carriers and mutation carriers with significant co-morbidity. Probably, the relevance of finding superficial T1a lesions varies between persons. The finding of two superficial T1a lesions during endoscopic surveillance in a 25-year-old healthy person may stimulate this person to start planning surgery within a few years. Whereas the finding of two superficial T1a lesions in endoscopic surveillance in a 65-year-old person with a history of a myocardial infarction may lead to the decision to continue endoscopic surveillance until there are reasons to think that there is development of a more invasive (≥T1a+) lesion.

In all, the most important lesions to detect in endoscopy are the T1+ lesions that tend to infiltrate deeper toward the submucosa. This is the moment that surgery should not be postponed unnecessarily as deeper infiltration is associated with an increased risk of metastasis.

Although endoscopists are of course aware of features of advanced diffuse gastric cancer, there is always a chance that even this can hide from endoscopic view. As such, there are some reports on interval cancers in *CDH1* mutation carriers under surveillance [6, 13]. Random biopsies may reveal hidden infiltrative diffuse gastric cancer, although negative biopsies unfortunately do not exclude the diagnosis because of the tendency of the tumor to grow in the submucosa.

13.4 Considerations on Biopsies (Random Versus Targeted) During Surveillance

As mentioned above, there is a large variation in the literature in the endoscopic detection of early DGC lesions (Table 13.1) [1–7, 14–18]. Despite the prescription of a 30-minute inspection to scan for abnormalities, most publications mention only detection of signet ring cells in random biopsies and not in targeted biopsies (Table 13.1). Probably, the great diversity among surveillance outcomes implicates a difference in the quality, experience and extensity of the surveillance endoscopy, and possibly also a diversity in penetrance between families. To perform endoscopy

with random biopsies no specific training in recognition of SRCC lesions is required and experience with surveillance of these patients is not essential. However, the low yield of SRCC lesions by random biopsies makes this approach less attractive. A model estimated that for a 90% detection rate, 1768 biopsies would be needed per patient to capture at least a single cancer focus [19]. A disadvantage of extensive random biopsies is the formation of scars. These scars can mimic the superficial pale SRCC lesions and widespread scars can therefore hamper adequate surveillance. However, the great diversity among surveillance outcomes in *CDH1* cohorts implicates that random biopsies cannot be omitted in the surveillance protocol, even in centers with considerable experience. Limiting the number of random biopsies in centers with high detection rates in targeted biopsies can be considered. Further development of endoscopic techniques to improve the yield of targeted biopsies is warranted. Hopefully, high-definition endoscopes, magnification, imaging-enhancing techniques, and artificial intelligence will help in further improving detection rates of SRCC foci in the near future.

13.5 Endoscopic Surveillance in *CTNNA1* Mutation Carriers

Only a limited number of families with germline *CTNNA* (encoding α-1-catenin) mutations have been reported, consequently, there is very minimal experience with endoscopic surveillance and it is difficult to make strong recommendations. Prophylactic gastrectomy approaches may be justified in families presenting with multiple gastric cancer cases. In a reported mutation carrier with a negative pre-operative endoscopy, the specimen did show a few mucosal signet ring cell foci [20]. However, by using good quality endoscopes and experience with endoscopic surveillance for diffuse-type gastric cancer, annual endoscopic surveillance in an expert HDGC center should be considered as is currently recommended by the International Gastric Cancer Linkage Consortium [8]. Prophylactic total gastrectomy can be considered depending on the results of endoscopy and biopsies and the penetrance of DGC in each family. Given the lack of knowledge about breast cancer in families carrying *CTNNA1* pathogenic variants, breast surveillance may be considered on a case-by-case basis [8].

13.6 What Is Known on Gastric Inlet Patches in Mutation Carriers?

In about 1:100 to 1:1000 gastroscopies a gastric inlet patch (GIP) is observed. A GIP is a patch consisting of ectopic columnar gastric mucosa with or without oxyntic glands. In case of the presence of a GIP, the relative frequency of an adenocarcinoma is higher in the GIP than outside the GIP [21]. In a small case series in eight carriers of a *CDH1* pathogenic germline variant 50% appeared to have a GIP. However, in biopsies of those no divergent gastric mucosa was found, thus no early signet cell lesions were observed [22]. To our knowledge, there are no reports on *CDH1*

mutation carriers who developed a clinically relevant carcinoma in a GIP. To increase the awareness and the knowledge on the possibility of SRCCs to develop in these GIPs, the current IGCLC guideline suggests inspection and biopsies if GIPs are present in mutation carriers.

13.7 What Is Known on *Helicobacter pylori* Infection in HDGC?

There is no clear association between *H. pylori* infections and the development of diffuse gastric cancer. The only clear association is the one between *H. pylori* infection and the development of intestinal gastric cancer, which is not associated with germline *CDH1*/ *CTNNA1* mutations. However, there are publications that report *H. pylori* infections in patients with advanced HDGC [3, 23]. Although these numbers are too small to draw firm conclusions, it may be hypothesized that *H. pylori* infection or chronic inflammation may trigger the development of invasive gastric cancer in *CDH1* mutation carriers.

13.8 Endoscopic Surveillance in HDGC-Like Families

In over 70% of all individuals who fulfill the criteria for genetic testing for HDGC, a *CDH1* or *CTNNA1* pathogenic germline variant cannot be detected. Therefore, for all cases fulfilling specific HDGC criteria, but without a *CDH1* or *CTNNA1* pathogenic germline variant, the term "HDGC-like group" was introduced in the guideline of 2020 [8]. Families that are considered to be HDGC-like fulfill HDGC genetic testing family criteria 1 or 2. These two criteria include: (1) two or more gastric cancer cases in a family regardless of age, with at least one confirmed DGC; or (2) the combination of at least one DGC at any age and one lobular breast cancer at age < 70 years, in different family members [8]. Next to these two criteria, there are too few data to support surveillance endoscopy in first-degree relatives of young individuals with DGC without any family history or pathogenic *CDH1*/*CTNNA1* variant.

For individuals from this HDGC-like group, data to support the optimal endoscopic screening remain scarce. The lifetime risk for HDGC-like first-degree family members is unknown, but estimated to be far lower compared to carriers of a *CDH1* or *CTNNA1* pathogenic germline variant. As such, a total prophylactic gastrectomy in family members for which the individual risk of developing DGC is unknown is not advised. Also, endoscopy data are limited. A few studies showed a 10-fold lower detection rate of early signet ring cell cancer lesions in this group compared to proven HDGC carriers in expert centers [7, 24]. In our study, we detected as a by-catch in 4% dysplasia and in 42% intestinal metaplasia—an estimated higher incidence than in the general population [24]. The meaning of this observation in this group is unclear, since both dysplasia and intestinal metaplasia are risk factors for the development of gastric cancer, but mostly associated with intestinal cancer

[25, 26]. Also, the optimal interval for endoscopic screening in this population is uncertain [24].

Based on this limited evidence, the current recommendation for first-degree relatives of HDGC-like affected individuals is to consider endoscopic screening on an annual basis in an expert center for at least 2 years. Surveillance is recommended to begin at 40 years of age, or 10 years prior to the youngest case of DGC within the family, with a minimum age of 18 years. The chance of a positive biopsy is probably highest during the first endoscopy, therefore after two negative endoscopies, as a patient-shared decision, an extension to every 2 or 3 years can be considered (Blair, Lancet Oncol 2020).

References

1. Shaw D et al (2005) Chromoendoscopic surveillance in hereditary diffuse gastric cancer: an alternative to prophylactic gastrectomy? Gut 54(4):461–468
2. Hebbard PC et al (2009) Prophylactic total gastrectomy (PTG) for hereditary diffuse gastric cancer (HDGC): the newfoundland experience with 23 patients. Ann Surg Oncol 16(7): 1890–1895
3. van Dieren JM et al (2020) Gastroscopic surveillance with targeted biopsies compared with random biopsies in CDH1 mutation carriers. Endoscopy 52(10):839–846
4. Vos EL et al (2020) Indications for Total gastrectomy in CDH1 mutation carriers and outcomes of risk-reducing minimally invasive and open gastrectomies. JAMA Surg 155(11):1050–1057
5. Jacobs MF et al (2019) Outcomes of endoscopic surveillance in individuals with genetic predisposition to hereditary diffuse gastric cancer. Gastroenterology 157(1):87–96
6. Friedman M et al (2021) Surveillance endoscopy in the management of hereditary diffuse gastric cancer syndrome. Clin Gastroenterol Hepatol 19(1):189–191
7. Mi EZ et al (2018) Comparative study of endoscopic surveillance in hereditary diffuse gastric cancer according to CDH1 mutation status. Gastrointest Endosc 87(2):408–418
8. Blair VR et al (2020) Hereditary diffuse gastric cancer: updated clinical practice guidelines. Lancet Oncol 21(8):e386–e397
9. Monster JL et al (2022) Diffuse gastric cancer: emerging mechanisms of tumor initiation and progression. Biochim Biophys Acta Rev Cancer 1877(3):188719
10. Al-Batran SE et al (2019) Perioperative chemotherapy with fluorouracil plus leucovorin, oxaliplatin, and docetaxel versus fluorouracil or capecitabine plus cisplatin and epirubicin for locally advanced, resectable gastric or gastro-oesophageal junction adenocarcinoma (FLOT4): a randomised, phase 2/3 trial. Lancet 393(10184):1948–1957
11. Roberts ME et al (2019) Comparison of CDH1 penetrance estimates in clinically ascertained families vs families ascertained for multiple gastric cancers. JAMA Oncol
12. Rocha JP et al (2018) Pathological features of total gastrectomy specimens from asymptomatic hereditary diffuse gastric cancer patients and implications for clinical management. Histopathology 73:878
13. Lim YC et al (2014) Prospective cohort study assessing outcomes of patients from families fulfilling criteria for hereditary diffuse gastric cancer undergoing endoscopic surveillance. Gastrointest Endosc 80(1):78–87
14. Chen Y et al (2011) A prospective study of total gastrectomy for CDH1-positive hereditary diffuse gastric cancer. Ann Surg Oncol 18(9):2594–2598
15. Pandalai PK et al (2011) Prophylactic total gastrectomy for individuals with germline CDH1 mutation. Surgery 149(3):347–355

16. Huneburg R et al (2016) Chromoendoscopy in combination with random biopsies does not improve detection of gastric cancer foci in CDH1 mutation positive patients. Endosc Int Open 4(12):E1305–e1310
17. Curtin BF et al (2021) Enhanced endoscopic detection of occult gastric cancer in carriers of pathogenic CDH1 variants. J Gastroenterol 56(2):139–146
18. Schueler SA et al (2021) Evaluation of confocal laser endomicroscopy for detection of occult gastric carcinoma in CDH1 variant carriers. J Gastrointest Oncol 12(2):216–225
19. Fujita H et al (2012) Endoscopic surveillance of patients with hereditary diffuse gastric cancer: biopsy recommendations after topographic distribution of cancer foci in a series of 10 CDH1-mutated gastrectomies. Am J Surg Pathol 36(11):1709–1717
20. Benusiglio PR et al (2019) Clinical implications of CTNNA1 germline mutations in asymptomatic carriers. Gastric Cancer 22(4):899–903
21. Orosey M, Amin M, Cappell MS (2018) A 14-year study of 398 esophageal adenocarcinomas diagnosed among 156,256 EGDs performed at two large hospitals: an inlet patch is proposed as a significant risk factor for proximal esophageal adenocarcinoma. Dig Dis Sci 63(2):452–465
22. Jadot V et al (2019) Hereditary diffuse gastric cancer : case series of 8 patients from a single family and literature review. Rev Med Liege 74(3):134–138
23. Gullo I et al (2018) Phenotypic heterogeneity of hereditary diffuse gastric cancer: report of a family with early-onset disease. Gastrointest Endosc 87(6):1566–1575
24. van der Post RS et al (2018) Outcomes of screening gastroscopy in first-degree relatives of patients fulfilling hereditary diffuse gastric cancer criteria. Gastrointest Endosc 87(2): 397–404 e2
25. Busuttil RA, Boussioutas A (2009) Intestinal metaplasia: a premalignant lesion involved in gastric carcinogenesis. J Gastroenterol Hepatol 24(2):193–201
26. de Vries AC et al (2008) Gastric cancer risk in patients with premalignant gastric lesions: a nationwide cohort study in the Netherlands. Gastroenterology 134(4):945–952

Lobular Carcinoma of the Breast: Spectrum of Imaging Findings and New Emerging Technologies on the Horizon

14

Anna Rotili, Luca Nicosia, Filippo Pesapane, Anna Bozzini, and Enrico Cassano

Abstract

Invasive lobular carcinoma (ILC) is the second most common histologic form of breast cancer, representing 5% to 15% of all invasive breast cancers. Due to the peculiar growth pattern, invasive lobular carcinoma remains clinically and radiologically challenging in many cases. Mammography has some well-known limitations in detecting ILC for the subtle findings related to its slow-growing and insinuating nature. Contrast-enhanced spectral mammography (CESM) is a new diagnostic method that enables the accurate detection of malignant breast lesions similar to that of breast MR. Breast magnetic resonance imaging (MR) is considered the most accurate imaging modality in detecting and staging invasive lobular carcinoma and it is strongly recommended in preoperative planning for all ILC.

14.1 Introduction

Invasive lobular breast cancer (ILC) is the second most common histologic type of invasive breast cancer behind invasive ductal carcinoma (IDC), accounting for 5%–15% of all invasive breast cancers [1].

The incidence of ILC is increasing steadily as a result of improved diagnostic techniques and the increased use of hormone replacement therapy in postmenopausal women [2–4].

Deficient E-cadherin expression, caused by an inactivation of the CDH1 gene, is an important immunohistochemical marker of ILC. E-cadherin is strongly related to cell-cell cohesion, and affects morphology and motility of cells. Therefore, loss of

A. Rotili (✉) · L. Nicosia · F. Pesapane · A. Bozzini · E. Cassano
Breast Radiology Division, European Institute of Oncology, IRCCS, Milan, Italy
e-mail: anna.rotili@ieo.it

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_14

E-cadherin expression may be the cause for the discohesive pattern of ILC growth, with single cells or files of cells invading the stroma with little effect on the overall tissue architecture [5]. Classic ILCs typically present low to intermediate mitotic index, low nuclear pleomorphism, and low histologic grade. They are generally hormone receptor-positive and rarely show HER2 protein overexpression or amplification. These features are generally associated with a good prognosis, yet some studies suggest that long-term outcomes of ILC are inferior to stage-matched invasive ductal carcinoma (IDC) [6]. Lobular tumors are significantly more likely to be Luminal A and have lower frequencies of TP53 pathway defects than ductal tumors [7].

In general, ILC tends to be more common among older and white women, more likely to be larger tumor size, and diagnosed at a later stage of disease as compared to IDC. Additionally, ILC is more often multifocal and bilateral [8–11].

The pathological diagnosis of ILC relies on the non-cohesive nature, single file or targetoid pattern of the cells, according to the fifth edition of the World Health Organization's Classification of Breast Tumours [12].

This peculiar growth pattern and its failure to elicit a desmoplastic response make ILC difficult to detect clinically, since lesions are often poorly circumscribed and fail to form discrete palpable masses and radiologically. In addition, current imaging modalities are not very specific to differentiate ILC from other invasive breast cancers, resulting in higher false-negative rates compared to other invasive breast cancers [13].

14.2 Imaging Findings of ILC

In the first evaluation of interval cancers after the initiation of breast cancer screening with mammography in the Netherlands, it became clear that ILC was a common pathologic diagnosis in the missed carcinoma group [14], attributed to the diffuse infiltrative pattern of the tumors and the poor desmoplastic reaction of the surrounding tissue.

The limitations of mammography in the detection and evaluation of invasive lobular carcinoma (ILC) have long been recognized. The sensitivity of mammograms in detecting ILC is about 57%–81%, with 35% of cases visible only on one view, more commonly on the cranio-caudal projection, and 30% of cases not visualized at all [15, 16].

Furthermore, it is well documented that mammographic sensitivity is inversely correlated with the degree of fibroglandular tissue density. When breast tissue is described as heterogeneous or extremely dense, the sensitivity of mammography for the detection of invasive tumors can be as low as 30–48% [17].

Berg et al. specifically examined the performance of mammography as a function of both tumor type and breast density. Mammographic sensitivity was 81% for IDC compared with 34% for ILC; when only those patients with dense breast tissue were considered, sensitivities decreased dramatically to 60% for IDC and 11% for ILC [18].

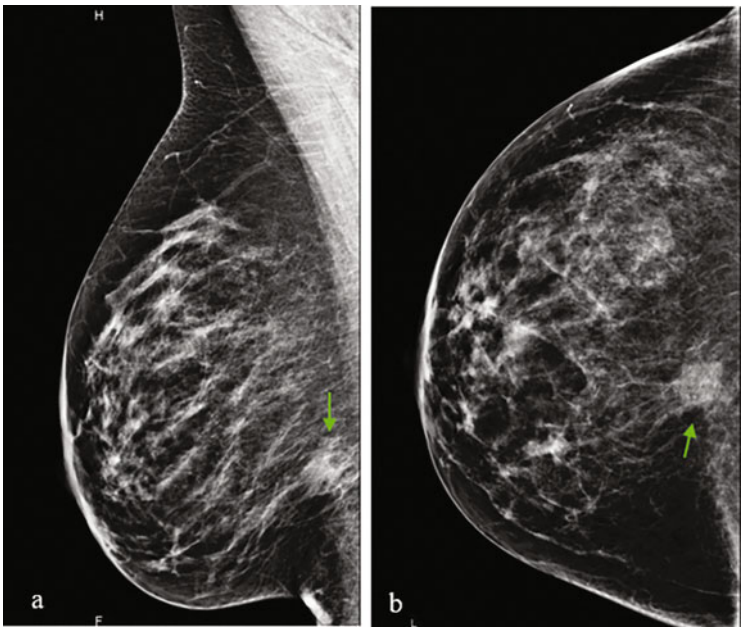


Fig. 14.1 Mass with spiculated and indistinct margins identified on mediolateral oblique and craniocaudal mammographic view. (**a**, **b**) Mediolateral oblique (**a**) and craniocaudal (**b**) views of the right breast show a low-density, irregular mass with spiculated margins (arrow) at the posterior depth in the inner-inferior quadrant of the right breast

Due to these diagnostic challenges, it is crucial for breast imaging radiologists to be aware of the atypical and subtle mammographic patterns of ILC.

The mammographic presentations of invasive lobular carcinoma include:

- A mass (up to 65% of cases) with irregular margins mostly speculated (Fig. 14.1) but sometimes circumscribed.
- Architectural distortion (10–34%) (Fig. 14.2) and asymmetric, focal density equal to or less than of normal breast parenchyma.
- Microcalcifications are uncommon (0 to 24%).
- Normal or benign mammographic findings in invasive lobular carcinoma are reported in 8 to 16% of cases.

In several series, it has been reported that up to 53% of ILC tumors present as spiculated masses on mammography [19, 20], while other investigators report that the majority of ILC tumors (68%) present as asymmetric densities or as masses with poorly defined margins [13]. All series report that a well-circumscribed mass is an uncommon mammographic presentation of ILC, seen in less than 1% of lobular tumors. Overall, the most common mammographic manifestations of ILC include spiculated, ill-defined masses, architectural distortion, and poorly defined asymmetric densities. The low sensitivity of mammography for detecting ILC is due to the

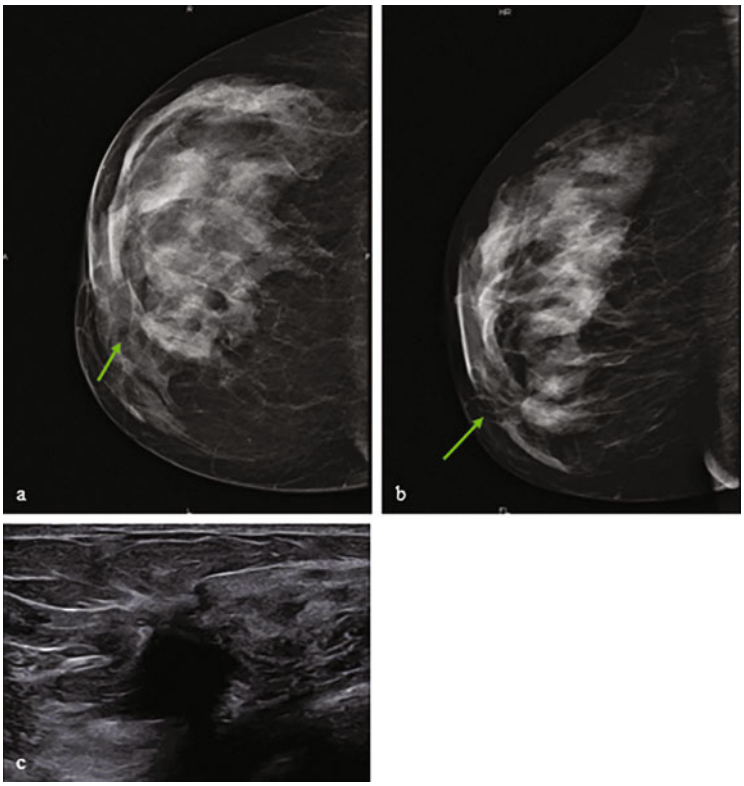


Fig. 14.2 Architectural distortion in a 57-year-old woman who presented with nipple retraction and focal hardness in the right breast. (**a**, **b**) Craniocaudal (**a**) and mediolateral (**b**) mammograms show an area of architectural distortion (arrow) in the inner-inferior quadrant of the right breast. (**c**) US shows an irregular, spiculated mass with posterior shadowing

low density of the tumor cells and lack of desmoplastic stromal reaction. Hilleren et al. [19] noted that 50% of spiculated ILC masses have an opacity less than or equal to that of normal breast parenchyma on all views obtained. Therefore, the morphology of the ILC tumor is not so much the problem as is the lack of contrast differences between ILC tumors and surrounding, and even overlapping, normal breast tissue. This allows these tumors to be camouflaged despite being in plain view on mammogram images. Architectural distortion accounts for approximately 14 to 25% of cases of mammographically detected ILC [19, 20]. Architectural distortion is identified on mammography when the normal architecture of the breast parenchyma is distorted but no discernable or discrete mass is obvious to the reader. It can include spicules radiating from a central point, as well as focal retraction or distortion of the edge of the parenchyma.

The ultimate goal of screening mammography programs worldwide is to detect breast cancer at an early stage. High-quality, high-resolution detailed images that exploit contrast differences between normal and diseased breast tissue are the

fundamental elements that allow detection of malignancy on mammograms. The accuracy of two-dimensional mammography (DM) can be improved by the use of breast tomosynthesis (DBT) or contrast-enhanced digital mammography (CEDM). DBT, digital breast tomosynthesis (DBT), also known as three-dimensional mammography, has the potential to explore breast tissues by producing thin slices of the mammographic view. DBT reduces the tissue-masking effect and improves lesion conspicuity with a better evaluation of parenchymal distortion, asymmetries, and ill-defined masses, which are common findings in ILC [21]. Several studies have shown that DBT has better capabilities than DM in lesion detection and characterization. The rate of detection of architectural distortions can improve up to 57%, when DBT was added to DM. Mass margins are better perceived, improving the evaluation of breast masses and allowing ill-defined margins to be appreciated as spiculated margins. The higher sensitivity of DBT improves the visibility of invasive lobular cancers, by more clearly depicting architectural distortions and speculations [22–25].

Contrast-enhanced mammography (CEM) is a relatively new technology in breast imaging which allows both a morphologic evaluation comparable to routine digital mammography and a simultaneous assessment of tumor neovascularity, similar to breast magnetic resonance (MR). CEDM generates a high-resolution, low-energy, full-field digital mammography image and a post-iodinated contrast recombined image to assess tumor neoangiogenesis. In the preoperative locoregional staging of ILC patients, the performance of CEDM outperforms standard DM in the evaluation of the extension of disease and in measurement of lesions, leading to improved surgical outcomes [26–28] (Fig. 14.3).

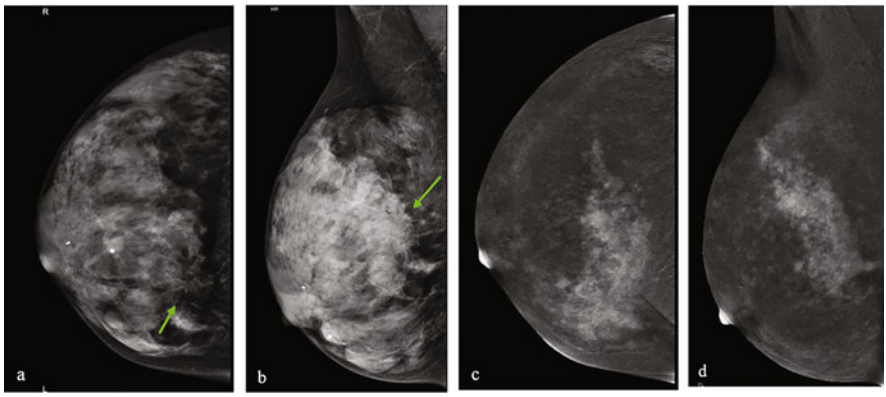


Fig. 14.3 Forty-seven-year-old woman with heterogeneously dense breast on routine mammography with architectural distortion in the right breast (arrow). (**a**, **b**) Craniocaudal and mediolateral MLO views show suspicious architectural distortion; extent is difficult to determine because of dense breast tissue. (**b**–**d**) Recombined image, after administration of iodinated contrast agent, demonstrates a suspicious non-mass enhancement. Patient underwent mastectomy. Histopathology demonstrated 6.5 cm of Grade 2 classic ILC. CEDM was more accurate than DM in assessing tumor extent

Breast magnetic resonance (MR) is the most sensitive tool for the diagnosis of ILC. Breast MR is routinely used in the preoperative staging to better determine the extent of newly diagnosed breast cancer and several medical societies, including the American Society of Breast Surgeons, the National Comprehensive Cancer Network and the European Society of Breast Imaging, recommend its use in the preoperative work-up of patients with ILC, particularly in women with dense breast. The retrospective sensitivity of breast MR imaging for ILC is high. In a meta-analysis of studies of MRI use in women with ILC, the overall sensitivity was 93.3% (95% CI 88–96%). Only few studies are prospective in design [29, 30].

The sensitivity of breast MR, in these prospective studies, ranged from 95 to 100% for ILC in line with the results from the retrospective studies. Furthermore, MRI is more likely to demonstrate an ILC span that is concordant with final surgical pathology size compared to conventional imaging which tends to underestimate the size of ILC and the T category of stage, the latter of which can affect treatment planning and clinical trial eligibility (Fig. 14.4).

Several studies suggest that preoperative MRI could provide particular value in the assessment of ILC by allowing better depiction of disease, particularly for women who are considering breast conservative surgery and for those with dense breast.

MRI findings of ILC include:

1. Solitary mass with irregular margins.
2. Multiple small enhancing foci connected by enhancing strands which correlate pathologically with non-contiguous tumor foci with malignant cells streaming in a single-file fashion in the breast stroma or enhancing clusters with non-enhancing intervening tissue which correspond to small tumor aggregates separated by normal tissue.
3. Areas of non-mass enhancement of various distributions and characteristics.

ILC generally shows longer time to peak enhancement, lower enhancement intensity, and less frequent wash-out kinetics than for IDC (Fig. 14.4), probably related to distinct patterns of vascular endothelial growth factor expression and angiogenesis [31–36].

Diffusion-weighted imaging (DWI) has been widely integrated into clinical practice for breast imaging. The major strength of DWI is that it can provide quantitative information about the motion of water molecules and biological characteristics of tumors without a contrast agent injection. Many studies have already demonstrated DWI as an imaging tool for improving breast cancer diagnosis and characterization [37–39]. DWI can help distinguish between benign and malignant lesions [40] and could reduce false positives and unnecessary biopsies [41, 42]. DWI can also be used to detect the early response to neoadjuvant chemotherapy (NAC) and in evaluating residual cancer after NAC [43, 44]. Therefore, DWI has the potential to be used as an unenhanced magnetic resonance imaging (MRI) for breast cancer screening [45, 46]. Jeong et al. reported that the visibility of ILC was lower compared to invasive carcinoma of NST on DWI, and mean ADC

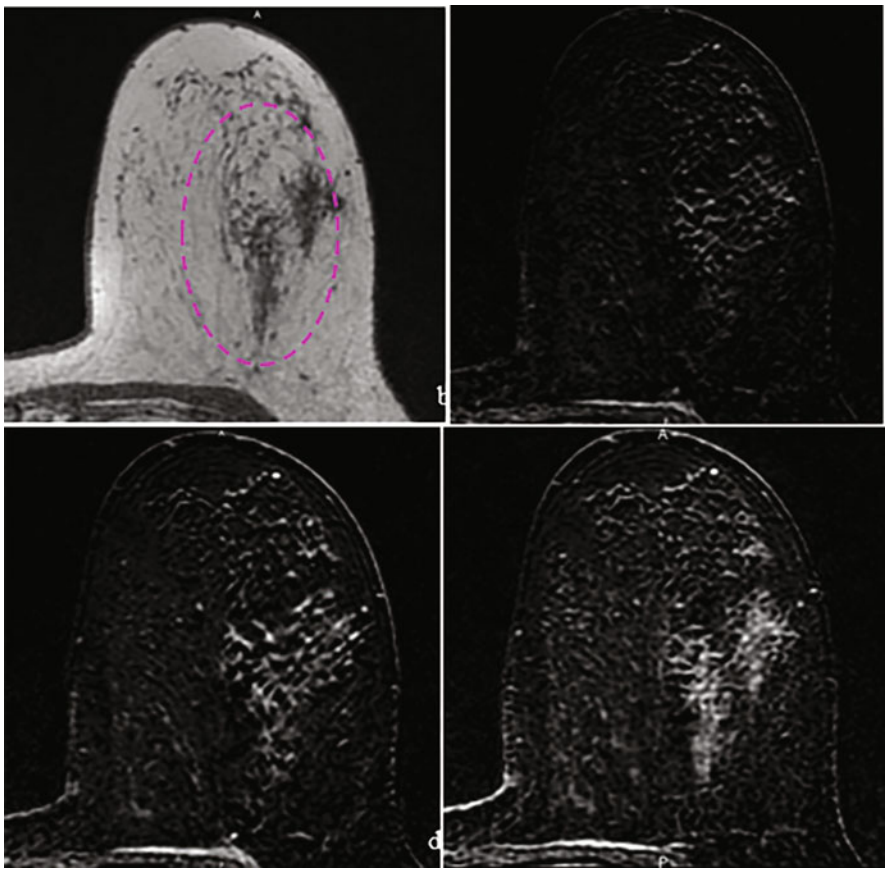


Fig. 14.4 Fifty-four-year old woman with a palpable lump on the left breast. (**a**) Axial T1-weighted image shows an architectural distortion (circle). (**b**–**d**) Subtracted images show a regional non-mass enhancement of low intensity on the first post-contrast dynamic sequence (**b**) and delayed maximum enhancement (**c**–**d**)

was higher in ILC than that of invasive carcinoma of NST, both data might be related to the peculiar infiltrative growth pattern of ILC responsible of low cellularity area within the tumor which would cause high ADC area. In addition, small ILC may cause a false-negative diagnosis on DWI [47]. In the literature, there are few studies on the role of DWI in the assessment of ILC; larger prospective studies are needed to prove the effectiveness of DWI in this pathology.

Note

Parts of this chapter are based on the open access publication by Johnson et al. (2015) [48].

The figures in this chapter are taken from personal archives.

References

1. Li CI, Anderson BO, Daling JR, Moe RE (2003) Trends in incidence rates of invasive lobular and ductal breast carcinoma. JAMA 289(11):1421. https://doi.org/10.1001/jama.289.11.1421
2. Rosenberg LU, Magnusson C, Lindström E et al (2006) Menopausal hormone therapy and other breast cancer risk factors in relation to the risk of different histological subtypes of breast cancer: a case-control study. Breast Cancer Res 8:R11. https://doi.org/10.1186/bcr1378
3. Biglia N, Mariani L, Sgro L, Mininanni P, Moggio G, Sismondi P (2007) Increased incidence of lobular breast cancer in women treated with hormone replacement therapy: implications for diagnosis, surgical and medical treatment. Endocr Relat Cancer 14(3):549–567. https://doi.org/10.1677/ERC-06-0060
4. Li CI, Weiss NS, Stanford JL, Daling JR (2000) Hormone replacement therapy in relation to risk of lobular and ductal breast carcinoma in middle-aged women. Cancer 88(11):2570–2577. https://doi.org/10.1002/1097-0142(20000601)88:11<2570::aid-cncr20>3.0.co;2-o
5. Hanby AM, Hughes TA (2008) In situ and invasive lobular neoplasia of the breast. Histopathology 52(1):58–66. https://doi.org/10.1111/j.1365-2559.2007.02891.x
6. Pestalozzi BC, Zahrieh D, Mallon E, Gusterson BA, Price KN, Gelber RD, Holmberg SB, Lindtner J, Snyder R, Thürlimann B, Murray E, Viale G, Castiglione-Gertsch M, Coates AS, Goldhirsch A, International Breast Cancer Study Group (2008) Distinct clinical and prognostic features of infiltrating lobular carcinoma of the breast: combined results of 15 International Breast Cancer Study Group clinical trials. J Clin Oncol 26(18):3006–3014. https://doi.org/10.1200/JCO.2007.14.9336
7. Ciriello G, Gatza ML, Beck AH, Wilkerson MD, Rhie SK, Pastore A, Zhang H, McLellan M, Yau C, Kandoth C, Bowlby R, Shen H, Hayat S, Fieldhouse R, Lester SC, Tse GM, Factor RE, Collins LC, Allison KH, Chen YY, Jensen K, Johnson NB, Oesterreich S, Mills GB, Cherniack AD, Robertson G, Benz C, Sander C, Laird PW, Hoadley KA, King TA, TCGA Research Network, Perou CM (2015) Comprehensive molecular portraits of invasive lobular breast cancer. Cell 163(2):506–519. https://doi.org/10.1016/j.cell.2015.09.033
8. Arpino G, Bardou VJ, Clark GM, Elledge RM (2004) Infiltrating lobular carcinoma of the breast: tumor characteristics and clinical outcome. Breast Cancer Res 6(3):R149–R156. https://doi.org/10.1186/bcr767
9. Li CI, Uribe DJ, Daling JR (2005) Clinical characteristics of different histologic types of breast cancer. Br J Cancer 93(9):1046–1052. https://doi.org/10.1038/sj.bjc.6602787
10. Williams LA, Hoadley KA, Nichols HB, Geradts J, Perou CM, Love MI, Olshan AF, Troester MA (2019) Differences in race, molecular and tumor characteristics among women diagnosed with invasive ductal and lobular breast carcinomas. Cancer Causes Control 30(1):31–39. https://doi.org/10.1007/s10552-018-1121-1
11. Biglia N, Maggiorotto F, Liberale V, Bounous VE, Sgro LG, Pecchio S, D'Alonzo M, Ponzone R (2013) Clinical-pathologic features, long term-outcome and surgical treatment in a large series of patients with invasive lobular carcinoma (ILC) and invasive ductal carcinoma (IDC). Eur J Surg Oncol 39(5):455–460. https://doi.org/10.1016/j.ejso.2013.02.007
12. Tan PH, Ellis I, Allison K, Brogi E, Fox SB, Lakhani S, Lazar AJ, Morris EA, Sahin A, Salgado R, Sapino A, Sasano H, Schnitt S, Sotiriou C, van Diest P, White VA, Lokuhetty D, Cree IA, WHO Classification of Tumours Editorial Board (2020) The 2019 World Health Organization classification of tumours of the breast. Histopathology 77(2):181–185. https://doi.org/10.1111/his.14091
13. Krecke KN, Gisvold JJ (1993) Invasive lobular carcinoma of the breast: mammographic findings and extent of disease at diagnosis in 184 patients. AJR Am J Roentgenol 161(5):957–960. https://doi.org/10.2214/ajr.161.5.8273634
14. Holland R, Mravunac M, Hendriks JH, Bekker BV (1982) So-called interval cancers of the breast. Pathologic and radiologic analysis of sixty-four cases. Cancer 49(12):2527–2533. https://doi.org/10.1002/1097-0142(19820615)49:12<2527::aid-cncr2820491220>3.0.co;2-e

15. Porter AJ, Evans EB, Foxcroft LM, Simpson PT, Lakhani SR (2014) Mammographic and ultrasound features of invasive lobular carcinoma of the breast. J Med Imaging Radiat Oncol 58(1):1–10. https://doi.org/10.1111/1754-9485.12080
16. Thomas M, Kelly ED, Abraham J, Kruse M (2019) Invasive lobular breast cancer: a review of pathogenesis, diagnosis, management, and future directions of early stage disease. Semin Oncol 46(2):121–132. https://doi.org/10.1053/j.seminoncol.2019.03.002
17. Mandelson MT, Oestreicher N, Porter PL, White D, Finder CA, Taplin SH, White E (2000) Breast density as a predictor of mammographic detection: comparison of interval- and screen-detected cancers. J Natl Cancer Inst 92(13):1081–1087. https://doi.org/10.1093/jnci/92.13.1081
18. Berg WA, Gutierrez L, NessAiver MS, Carter WB, Bhargavan M, Lewis RS, Ioffe OB (2004) Diagnostic accuracy of mammography, clinical examination, US, and MR imaging in preoperative assessment of breast cancer. Radiology 233(3):830–849. https://doi.org/10.1148/radiol.2333031484
19. Hilleren DJ, Andersson IT, Lindholm K, Linnell FS (1991) Invasive lobular carcinoma: mammographic findings in a 10-year experience. Radiology 178(1):149–154. https://doi.org/10.1148/radiology.178.1.1984294
20. Le Gal M, Ollivier L, Asselain B, Meunier M, Laurent M, Vielh P, Neuenschwander S (1992) Mammographic features of 455 invasive lobular carcinomas. Radiology 185(3):705–708. https://doi.org/10.1148/radiology.185.3.1438749
21. Helvie MA (2010) Digital mammography imaging: breast tomosynthesis and advanced applications. Radiol Clin N Am 48(5):917–929. https://doi.org/10.1016/j.rcl.2010.06.009
22. Grubstein A, Rapson Y, Morgenstern S, Gadiel I, Haboosheh A, Yerushalmi R, Cohen M (2016) Invasive lobular carcinoma of the breast: appearance on digital breast tomosynthesis. Breast Care (Basel) 11(5):359–362. https://doi.org/10.1159/000450868
23. Korhonen KE, Conant EF, Cohen EA, Synnestvedt M, McDonald ES, Weinstein SP (2019) Breast cancer conspicuity on simultaneously acquired digital mammographic images versus digital breast tomosynthesis images. Radiology 292(1):69–76. https://doi.org/10.1148/radiol.2019182027
24. Chamming's F, Kao E, Aldis A, Ferré R, Omeroglu A, Reinhold C, Mesurolle B (2017) Imaging features and conspicuity of invasive lobular carcinomas on digital breast tomosynthesis. Br J Radiol 90(1073):20170128. https://doi.org/10.1259/bjr.20170128
25. Romanucci G, Zantedeschi L, Ventriglia et al (2021) Lobular breast cancer conspicuity on digital breast tomosynthesis compared to synthesized 2D mammography: a multireader study. J Imaging 7:185. https://doi.org/10.3390/jimaging7090185
26. Patel BK, Davis J, Ferraro C, Kosiorek H, Hasselbach K, Ocal T, Pockaj B (2018) Value added of preoperative contrast-enhanced digital mammography in patients with invasive lobular carcinoma of the breast. Clin Breast Cancer 18(6):e1339–e1345. https://doi.org/10.1016/j.clbc.2018.07.012
27. Amato F, Bicchierai G, Cirone D, Depretto C, Di Naro F, Vanzi E, Scaperrotta G, Bartolotta TV, Miele V, Nori J (2019) Preoperative loco-regional staging of invasive lobular carcinoma with contrast-enhanced digital mammography (CEDM). Radiol Med 124(12):1229–1237. https://doi.org/10.1007/s11547-019-01116-7
28. Patel BK, Garza SA, Eversman S, Lopez-Alvarez Y, Kosiorek H, Pockaj BA (2017) Assessing tumor extent on contrast-enhanced spectral mammography versus full-field digital mammography and ultrasound. Clin Imaging 46:78–84. https://doi.org/10.1016/j.clinimag.2017.07.001
29. Francis A, England DW, Rowlands DC, Wadley M, Walker C, Bradley SA (2001) The diagnosis of invasive lobular breast carcinoma. Does MRI have a role? Breast 10(1):38–40. https://doi.org/10.1054/brst.2000.0183
30. Caramella T, Chapellier C, Ettore F, Raoust I, Chamorey E, Balu-Maestro C (2007) Value of MRI in the surgical planning of invasive lobular breast carcinoma: a prospective and a retrospective study of 57 cases: comparison with physical examination, conventional imaging, and histology. Clin Imaging 31(3):155–161. https://doi.org/10.1016/j.clinimag.2007.01.001

31. Yeh ED, Slanetz PJ, Edmister WB, Talele A, Monticciolo D, Kopans DB (2003) Invasive lobular carcinoma: spectrum of enhancement and morphology on magnetic resonance imaging. Breast J 9(1):13–18. https://doi.org/10.1046/j.1524-4741.2003.09104.x
32. Hovis KK, Lee JM, Hippe DS, Linden H, Flanagan MR, Kilgore MR, Yee J, Partridge SC, Rahbar H (2021) Accuracy of preoperative breast MRI versus conventional imaging in measuring pathologic extent of invasive lobular carcinoma. J Breast Imaging 3(3):288–298. https://doi.org/10.1093/jbi/wbab015
33. Mann RM, Hoogeveen YL, Blickman JG et al (2008) MRI compared to conventional diagnostic work-up in the detection and evaluation of invasive lobular carcinoma of the breast: a review of existing literature. Breast Cancer Res Treat 107:1–14. https://doi.org/10.1007/s10549-007-9528-5
34. Mann RM (2010) The effectiveness of MR imaging in the assessment of invasive lobular carcinoma of the breast. Magn Reson Imaging Clin N Am 18(2):259–76, ix. https://doi.org/10.1016/j.mric.2010.02.005
35. Amin AL, Sack S, Larson KE, Winblad O, Balanoff CR, Nazir N, Wagner JL (2021) Does the addition of breast MRI add value to the diagnostic workup of invasive lobular carcinoma? J Surg Res 257:144–152. https://doi.org/10.1016/j.jss.2020.07.035
36. Lee AH, Dublin EA, Bobrow LG, Poulsom R (1998) Invasive lobular and invasive ductal carcinoma of the breast show distinct patterns of vascular endothelial growth factor expression and angiogenesis. J Pathol 185(4):394–401. https://doi.org/10.1002/(SICI)1096-9896(199808)185:4<394::AID-PATH117>3.0.CO;2-S
37. Yabuuchi H, Matsuo Y, Sunami S, Kamitani T, Kawanami S, Setoguchi T, Sakai S, Hatakenaka M, Kubo M, Tokunaga E, Yamamoto H, Honda H (2011) Detection of non-palpable breast cancer in asymptomatic women by using unenhanced diffusion-weighted and T2-weighted MR imaging: comparison with mammography and dynamic contrast-enhanced MR imaging. Eur Radiol 21(1):11–17. https://doi.org/10.1007/s00330-010-1890-8
38. Kazama T, Kuroki Y, Kikuchi M, Sato Y, Nagashima T, Miyazawa Y, Sakakibara M, Kaneoya K, Makimoto Y, Hashimoto H, Motoori K, Takano H (2012) Diffusion-weighted MRI as an adjunct to mammography in women under 50 years of age: an initial study. J Magn Reson Imaging 36(1):139–144. https://doi.org/10.1002/jmri.23626
39. McDonald ES, Hammersley JA, Chou S-HS, Rahbar H, Scheel JR, Lee CI, Liu C-L, Lehman CD, Partridge SC (2016) Performance of DWI as a rapid unenhanced technique for detecting mammographically occult breast cancer in elevated-risk women with dense breasts. Am J Roentgenol 207(1):205–216. https://doi.org/10.2214/AJR.15.15873
40. Dorrius MD, Dijkstra H, Oudkerk M, Sijens PE (2014) Effect of b value and pre-admission of contrast on diagnostic accuracy of 1.5-T breast DWI: a systematic review and meta-analysis. Eur Radiol 24(11):2835–2847. https://doi.org/10.1007/s00330-014-3338-z
41. Spick C, Pinker-Domenig K, Rudas M, Helbich TH, Baltzer PA (2014) MRI-only lesions: application of diffusion-weighted imaging obviates unnecessary MR-guided breast biopsies. Eur Radiol 24(6):1204–1210. https://doi.org/10.1007/s00330-014-3153-6
42. Rahbar H, Zhang Z, Chenevert TL, Romanoff J, Kitsch AE, Hanna LG, Harvey SM, Moy L, DeMartini WB, Dogan B, Yang WT, Wang LC, Joe BN, Oh KY, Neal CH, McDonald ES, Schnall MD, Lehman CD, Comstock CE, Partridge SC (2019) Utility of diffusion-weighted imaging to decrease unnecessary biopsies prompted by breast MRI: a trial of the ECOG-ACRIN cancer research group (A6702). Clin Cancer Res 25(6):1756–1765. https://doi.org/10.1158/1078-0432.CCR-18-2967
43. Park SH, Moon WK, Cho N, Song IC, Chang JM, Park I-A, Han W, Noh D-Y (2010) Diffusion-weighted MR imaging: pretreatment prediction of response to neoadjuvant chemotherapy in patients with breast cancer. Radiology 257(1):56–63. https://doi.org/10.1148/radiol.10092021
44. Wu L-M, Hu J-N, Gu H-Y, Hua J, Chen J, Xu J-R (2012) Can diffusion-weighted MR imaging and contrast-enhanced MR imaging precisely evaluate and predict pathological response to neoadjuvant chemotherapy in patients with breast cancer? Breast Cancer Res Treat 135(1):17–28. https://doi.org/10.1007/s10549-012-2033-5

45. Partridge SC, Nissan N, Rahbar H, Kitsch AE, Sigmund EE (2017) Diffusion-weighted breast MRI: clinical applications and emerging techniques. J Magn Reson Imaging 45(2): 337–355. https://doi.org/10.1002/jmri.25479
46. Rotili A, Trimboli RM, Penco S, Pesapane F, Tantrige P, Cassano E, Sardanelli F (2020) Double reading of diffusion-weighted magnetic resonance imaging for breast cancer detection. Breast Cancer Res Treat 180(1):111–120. https://doi.org/10.1007/s10549-019-05519-y
47. Jeong S, Kim TH (2022) Diffusion-weighted imaging of breast invasive lobular carcinoma: comparison with invasive carcinoma of no special type using a histogram analysis. Quant Imaging Med Surg 12(1):95–105. https://doi.org/10.21037/qims-21-355
48. Johnson K, Sarma D, Hwang ES (2015) Lobular breast cancer series: imaging. Breast Cancer Res 17(1):94. https://doi.org/10.1186/s13058-015-0605-0

Part V

Surgery

Prophylactic Total Gastrectomy: Techniques

15

Maria Bencivenga, Giovanni De Manzoni, and Franco Roviello

Abstract

Prophylactic total gastrectomy (PTG) represents the treatment of choice for carriers of pathogenetic *CDH1* mutations in the context of hereditary diffuse gastric cancer (HDGC) syndrome. PTG consists in a total gastrectomy: it could be performed both through open or minimally invasive surgery depending on the surgeon's expertise, but the higher rate of postoperative complications reported after laparoscopic procedures should be taken into account especially in the specific context of a prophylactic surgery. The intra-operative control of resection margins with frozen section is mandatory to exclude residual of gastric mucosa. D1/D1+ is suggested but D2 dissection can be considered. Roux-en-Y reconstruction is preferred. Long-term follow-up shows a good quality of life after this type of surgery despite the weight loss.

15.1 Introduction

Hereditary diffuse gastric cancer (HDGC) is the most common hereditary form of gastric cancer.

It represents an autosomal dominant syndrome determined by the presence of pathogenic germline mutations of *CDH1* gene, codifying for the membrane protein E-cadherin, in either an isolated individual with diffuse gastric cancer (DGC), or in a family with one or more DGC cases in first-degree or second-degree relatives.

M. Bencivenga (✉) · G. De Manzoni
General and Upper GI Surgery Division, University of Verona, Verona, Italy
e-mail: maria.bencivenga@univr.it

F. Roviello
Unit of General Surgery and Surgical Oncology, Department of Medicine, Surgery and Neuroscience, University of Siena, Siena, Italy

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_15

According to the new guidelines, mutations in a second gene encoding for another membrane protein, the α-catenin (CTNNA1), also play a role in the development of this inherited syndrome [1].

The classification of the pathogenic impact of different types of germline *CDH1* has recently been reported by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [2]. The classes IV and V of *CDH1* variants are considered pathogenic or likely pathogenic variants (P/LP) and they include nonsense, missense, frameshift, splice sites, single exon or multi-exon deletions: these are the mutations included in the definition of HDGC [2]. Other types of *CDH1* gene variants are classified as variants of unknown significance (VUS). Subjects harboring such VUS together with the cases characterized by a familial aggregation of diffuse gastric cancer without, however, the identification of pathogenic variants of the *CDH1* and *CTNNA1* genes, of note, are included in the new category of HDGC-like [1].

Prophylactic total gastrectomy (PTG) represents the treatment of choice for carriers of pathogenic/likely pathogenic *CDH1* mutations, because of the lack of effective endoscopic screening and surveillance programs.

In fact, the global accuracy of the Cambridge Protocol, the most frequently used protocol for endoscopic surveillance varies from 28% to 43% and these values become even lower in case of asymptomatic carriers (about 26%). Other endoscopic protocols have been introduced (such as the Bethesda Protocol), with the aim to decrease the false negative cases, but they are more challenging to perform because of the high number of biopsies required (almost 90).

Similar to many other studies in the past, a recent cohort study [3] shows the results of 15 years of experience (2006–2020) at Memorial Sloan Kettering Cancer Center. A total of 78 asymptomatic patients with pathogenic or likely pathogenic (P/LP) germline *CDH1* variants and a completely negative preoperative endoscopy underwent total gastrectomy. In these patients, signet ring cells (SRC) foci were detected in surgical specimen in 66 cases (85%) and in 12 (15%) did not. The majority of them present the involvement of mucosa (pT1a). These data underline the poor accuracy of endoscopic surveillance protocols suggesting that at time, PTG represents the only safe risk reduction strategy.

Of note, the International Gastric Cancer Linkage Consortium (IGCLC) [1] recommended gastric surveillance instead of a PTG in HDGC-like families.

15.2 Timing

The optimal timing for PTG is unclear; International Gastric Cancer Linkage Consortium recommends the procedure between the age of 20 and 30, after a multidisciplinary consultation. PTG should be avoided before age of 20 and in patients older than 70, considering the high morbidity and mortality rates of surgery.

The global distribution of PTG was recently analyzed by a systematic review published in 2022 [4]. A total of 224 surgical procedures classified as PTG were identified, with an age range of 18–71 years. The majority of PTGs were performed

in the USA (112 [50%]) followed by the Netherlands (40 [17.8%]), Canada (28 [12.5%]), Belgium (8 [3.6%]), Spain (8 [3.6%]), Denmark (7 [3.1%]), Portugal (6 [2.7%]), Austria (6 [2.7%]), Mexico (4 [1.8%)]), Iran (2 [0.9%]), Australia (1 [0.4%]), Chile (1 [0.4%]), Germany (1 [0.4%)], and Italy [1 (0.4%)], respectively.

In 219 PTG cases, histology data were available. In 32 (14.6%) cases "no cancer" was detected after PTG, in 159 (72.6%) cases at least one focus of SRC was identified and in 28 (12.8%) cases an invasive tumor was detected, unfortunately what is meant with invasive tumor was not specified. The higher frequency of "no cancer" detections after histopathology examination was reported from the USA group (19.6%) in comparison to the others, probably according to the low incidence of Gastric Cancer in this area [5].

15.3 Technique

Technically PTG can be performed either open or minimally invasive (laparoscopic or robot-assisted) based on the experience of surgeon.

However, it should be reminded that laparoscopic total gastrectomy (LATG) remains a technically demanding procedure with unsolved safety issues even in Eastern countries.

Indeed, in a retrospective study by Kodera et al. [6], data from 11,740 clinical stage I gastric cancer patients treated throughout Japan were collected, 7793 of whom underwent open and 3974 laparoscopic surgery. A propensity score-matched analysis was performed between the two groups. Of note, the incidence of anastomotic leakage reached a significant difference, being lower in open compared to laparoscopic surgery (3.6% in OTG vs. 5.4% in LATG, $p < 0.001$).

Moreover, although the length of hospital stay was significantly longer in the open surgery group, the incidence of readmission and reoperation within 30 days after gastrectomy was higher in the laparoscopic group (2.7% vs. 1.7% for readmission, $p = 0.002$, and 4.5% vs. 3.3% for reoperation, $p = 0.009$) [6]. These retrospective results from a nationwide survey raise some doubts about surgical safety in an unselected population.

In another study, the phase II KLASS-03 trial, with a more selected population, including 160 patients undergoing LATG, the complication rate was 20.6%, which is comparable to historical controls, 15 patients (9.4%) exhibited grade III or higher complications according to the Clavien-Dindo classification, and anastomotic leakage was present in only three patients (1.9%) [7].

The authors investigated whether the anastomotic technique of esophagojejunal anastomoses (45 extracorporeal circular stapled, 64 intracorporeal circular stapled, and 51 intracorporeal linear stapled anastomosis) impacts the incidence of postoperative complications. Early postoperative complications were similar between groups but long-term complications, specifically esophagojejunostomy stenoses were significantly more frequent in the intracorporeal circular stapling group [8].

Taking together the evidence from the Japanese survey and the Korean trial, LATG is still associated with esophagojejunal anastomosis issues such as leakages

or stenosis, and these findings should be considered when planning the surgical strategy especially in this specific context of PTG.

Indeed, PTG have same specific technical issues: the operation both for open and laparoscopic approaches follows the steps already described for standard procedures [9, 10], including a demolitive and a subsequent reconstructive phase.

As in a standard total gastrectomy, the demolitive phase includes the following steps:

- Colo-epiploic detachment and access to the retroactivity of the epiploons; the dissection continues along the greater curvature up to the left gastroepiloic pedicle that is ligated at its origin with complete removal of nodes at Station 4sd, then the gastrosplenic ligament is separated up to the left side of the esophageal hiatus by dividing the short gastric vessels from the surface of the spleen to dissect the nodes at Stations 4sa and 2 and to mobilize the upper part of the greater curvature of the stomach.
- The right gastroepiploic vessels are then dissected en bloc with lymphatic tissue (Station 6). The lesser omentum is then opened from pars faccida to the hepatic pedicle. With this dissection, the lymph nodes near the lesser curvature are removed (Station 3). Next, the proper hepatic artery is cleaned to identify the right gastric artery. This maneuver allows to dissect the lymph nodes of Station 5. Then, the release of the first part of the duodenum is completed and its transection can be performed.
- Dissection of the gastro-pancreatic folder and ligation of the left gastric artery and vein at level of upper margin of the pancreas allows the dissection of Station 7.
- Division of the phrenoesophageal membrane (LaimerBertelli membrane) and the section of the two vagus nerves leads to the release of cardia and liberation of abdominal esophagus, allowing a complete en bloc removal of right para-cardial nodes and of nodes along the first branch of left gastric artery (Station 1).

In the specific context of a PTG, the proximal section should be performed about 3 cm above the gastroesophageal junction and a frozen section of the proximal margin has to be checked intraoperatively by the pathologist in order to exclude residual gastric mucosa before moving on to the reconstructive phase.

In case of residual gastric mucosa, an additional proximal section has to be performed: this further impacts on technical challenges of performing esophago-jejunal anastomosis through laparoscopic approach in PTG.

To this regard, a Dutch retrospective study [11] reports that in 25 of 26 patients undergoing PTG, an intraoperative frozen-section examination of the proximal resection margin was used to verify the complete removal of gastric mucosa. In 9 of these patients (36%) proximal resection presented residual of gastric mucosa at the pathological examination so that a new resection was performed.

In the Memorial Sloan Kettering Cancer Center cohort [3] 57 of 101 asymptomatic patients had frozen section of the proximal margin during surgical operation, all of which ultimately contained squamous mucosa. Of the remaining 44 patients who had only a gross examination of the proximal margin without intraoperative frozen

section confirmation, 3 (6.8%) ultimately had gastric mucosa ($n = 2$) or SRCC ($n = 1$) in the final proximal margin. Unfortunately, a distinction between open and minimally invasive approaches was not reported in this study while it would be of help to understand the impact of the laparoscopic approach on proximal margin status.

In the near future, robotic surgery would appear to be a good option for these cases. Evidence is accumulating [12, 13] on a low complication rate, especially at the anastomotic level, when comparing laparoscopic and robotic approaches.

An umbrella review [14] highlights that RG has a longer operation time compared to laparoscopic gastrectomy but inferior blood loss, reduction in hospital stay, and a more rapid recovery of bowel function. Moreover, there was no difference in terms of total complication rate, mortality, morbidity, anastomotic leakage, anastomotic stenosis, intestinal obstruction, and in conversion rate to open technique.

Of note, an interesting finding is the association of robotic gastrectomy with a greater ability to perform a wider proximal margin: this assumes a value especially for PTGs.

Usually, in open surgery the anastomosis is performed with a circular stapler, the same is done in minimally invasive robot-assisted gastrectomy by introducing the stapler through a mini-laparotomy, while in laparoscopic total gastrectomy, different anastomotic techniques are reported.

15.4 Lymphaadenectomy

In case of PTG, a D1/D1 + lymphadenectomy is indicated, but also a D2 lymphadenectomy could be considered.

According to Japanese Guidelines [15] in case of a total gastrectomy, D1 + lymphadenectomy includes Stations 1 to 7 (as a D1 dissection) and stations 8a, 9, and 11p. A D2 lymphadenectomy, to be correctly performed, in addition to the D1 stations (1 to 7) includes Stations 8a, 9, 11p, 11d, and 12a.

In case of HDGC, the presence of submucosal invading foci (pT1b) cannot be excluded, and these have a not negligible frequency of positive lymph nodes (about 17–25%).

Looking at the risk of lymph node metastases in early gastric cancer (EGC), a previous study by our group [16] by analyzing a total of 652 cases of resected EGC from three Italian surgical centers with high experience in gastric cancer surgery (>30 cases/year) reported an incidence of lymph node metastases significantly higher in tumors penetrating the submucosa ($P < 0.001$) and in the diffuse/mixed tumors according to Lauren ($P < 0.001$). The multivariate analyses confirmed that submucosa invasion and Lauren diffuse/mixed type were independent predictors of nodal involvement.

A recent metanalysis [17] reporting the incidence of lymph nodes metastases in EGC according to standard and extended criteria generally used for endoscopic resection showed that cases which could be classified in the expanded criteria due

to undifferentiated histological type presented a higher incidence of lymph nodes metastases compared to other mucosal EGC (about 2.6% vs. 0.11%).

These data confirm that histological type (especially diffuse type) and submucosal invasion are risk factors for an increased incidence of lymph node metastases. This should be considered also when managing HDGC syndrome, in asymptomatic patients or with negative endoscopic surveillance, given to the high rate of presence of tumor foci found on the surgical specimens and the possibility that these can invade the submucosa.

There are some anatomical landmarks that guide the execution of the lymphadenectomy [10]:

- Skeletonization of the celiac trunk in the tract included among the origin of the common hepatic artery and the splenic artery allows the dissection of lymph nodes of Station 9.
- Section of the posterior parietal peritoneum along the superior margin of the pancreas in order to skeletonize the common hepatic artery until the origin of the gastroduodenal trunk, allow the complete dissection of nodal Station 8a.
- Skeletonization of the splenic artery leftward in its proximal tract until the origin of the posterior gastric artery (Station 11p) and around the splenic artery to the tip of the pancreas tail (Station 11d).
- Extending rightward the dissection of the proper hepatic artery the hepatoduodenal ligament allows the removal of the anterior lymph nodes of the hepato-duodenal ligament (Station 12a); this dissection can be considered complete if it is conducted until the portal vein becomes visible.

15.5 Other Issues

Regarding the reconstruction technique, a Roux-en-Y is generally preferred with the aim to reduce bile reflux. A jejunal pouch reconstruction has been suggested by some surgeons but there are no clear data indicating advantages of this more complex technique [18].

Furthermore, during the operation, it is necessary to check the presence or not of Meckel's diverticulum, common site of gastric heterotopia, and, if present, remove it.

After performing a PTG, the pathological analysis of the surgical specimen must be guaranteed by an expert pathologist. Furthermore, as suggested by the guidelines [1] the entire stomach should be analyzed, with many slices, to identify all microscopic foci. A total gastric mapping requires approximately 120–270 blocks, with up to three slices per block.

This underlines the importance of centralization of PTGs not only for the surgical aspects that are discussed above, but also for the pathological analysis which can be really complex from an organizational point of view.

15.6 Adverse Effects

As for total gastrectomy performed for sporadic gastric cancer, PTG is also associated with several side effects, such as early and late dumping syndrome, malabsorption, and postprandial fullness. This procedure in fact is associated with the decrease of vitamin B12 and protein absorption, bacterial overgrowth due to loss of parietal and chief cells of the stomach, reflux, dumping, and weight loss [19]. Of note, PTG is usually performed in young people with a long-life expectancy and needs such as maternity, therefore nutritional support is essential specifically after PTG.

A recent study [20] on a cohort of American patients, shows the long-term results after PTG. Each patient had significant weight loss (mean 23%) but all had a normal body mass index. In total, 40% had bile reflux gastritis controlled with sucralfate. Each returned to work and, if given the choice, said that they would undergo the surgery again.

15.7 Conclusions

In conclusion, PTG is currently the safest indication in patients carrying pathogenic *CDH1* mutations. However, since after a PTG often the neoplastic foci found on the surgical specimen are early and limited to the mucosa, the biggest challenge for the future is to understand if it is possible to more accurately determine the risk of neoplastic progression of gastric foci, based on the characteristics of *CDH1* mutation and molecular analyses carried out on tumors samples from the same family with the final aim to indicate PTG only in selected cases.

References

1. Blair VR, McLeod M, Carneiro F et al (2020) Hereditary diffuse gastric cancer: updated clinical practice guidelines. Lancet Oncol 21(8):e386–e397. https://doi.org/10.1016/S1470-2045(20)30219-9
2. Richards S, Aziz N, Bale S et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17(5):405–424. https://doi.org/10.1038/gim.2015.30
3. Vos EL, Salo-Mullen EE, Tang LH, et al (2020) Indications for total gastrectomy in CDH1 mutation carriers and outcomes of risk-reducing minimally invasive and open Gastrectomies. JAMA Surg 155(11):1050–1057. https://doi.org/10.1001/jamasurg.2020.3356
4. Corso G, Magnoni F, Nicastro V et al (2022) Global distribution of prophylactic total gastrectomy in E-cadherin (CDH1) mutations. Semin Oncol 49(2):130–135. https://doi.org/10.1053/j.seminoncol.2022.03.001
5. Corso G, Marrelli D, Pascale V et al (2012) Frequency of CDH1 germline mutations in gastric carcinoma coming from high- and low-risk areas: metanalysis and systematic review of the literature. BMC Cancer 12:8. https://doi.org/10.1186/1471-2407-12-8

6. Kodera Y, Yoshida K, Kumamaru H et al (2019) Introducing laparoscopic total gastrectomy for gastric cancer in general practice: a retrospective cohort study based on a nationwide registry database in Japan. Gastric Cancer 22(1):202–213. https://doi.org/10.1007/s10120-018-0795-0
7. Hyung WJ, Yang HK, Han SU et al (2019) A feasibility study of laparoscopic total gastrectomy for clinical stage I gastric cancer: a prospective multi-center phase II clinical trial, KLASS 03. Gastric Cancer 22(1):214–222. https://doi.org/10.1007/s10120-018-0864-4
8. Yang HK, Hyung WJ, Han SU et al (2021) Comparison of surgical outcomes among different methods of esophagojejunostomy in laparoscopic total gastrectomy for clinical stage I proximal gastric cancer: results of a single-arm multicenter phase II clinical trial in Korea, KLASS 03. Surg Endosc 35(3):1156–1163. https://doi.org/10.1007/s00464-020-07480-0
9. de Manzoni G, Roviello F, Siquini W (eds) (2012) Surgery in multimodal treatment of gastric cancer. Springer, Milano. https://doi.org/10.1007/978-88-470-2318-5
10. Giacopuzzi S, Bencivenga M, Cipollari C et al (2017) Lymphadenectomy: how to do it? Transl Gastroenterol Hepatol 2:28. https://doi.org/10.21037/tgh.2017.03.09
11. van der Kaaij RT, van Kessel JP, van Dieren JM et al (2018) Outcomes after prophylactic gastrectomy for hereditary diffuse gastric cancer. Br J Surg 105(2):e176–e182. https://doi.org/10.1002/bjs.10754
12. Kumamoto T, Ishida Y, Igeta M, Hojo Y, Nakamura T, Kurahashi Y, Shinohara H (2022) Potential advantages of robotic total gastrectomy for gastric cancer: a retrospective comparative cohort study. J Robot Surg 16(4):959–966. https://doi.org/10.1007/s11701-021-01328-y
13. Marano L, D'Ignazio A, Resca et al (2021) Robotic-assisted gastrectomy for gastric cancer: single Western center results. Updates Surg 73(3):865–872. https://doi.org/10.1007/s13304-020-00896-2
14. Marano L, Fusario D, Savelli V, Marrelli D, Roviello F (2021) Robotic versus laparoscopic gastrectomy for gastric cancer: an umbrella review of systematic reviews and meta-analyses. Updates Surg 73(5):1673–1689. https://doi.org/10.1007/s13304-021-01059-7
15. Japanese Gastric Cancer Association (2021) Japanese gastric cancer treatment guidelines 2018 (5th edition), Gastric Cancer. 24(1):1–21. https://doi.org/10.1007/s10120-020-01042-y
16. Roviello F, Rossi S, Marrelli D et al (2006) Number of lymph node metastases and its prognostic significance in early gastric cancer: a multicenter Italian study. J Surg Oncol 94(4):275–280; discussion 274. https://doi.org/10.1002/jso.20566
17. Abdelfatah MM, Barakat M, Lee H et al (2018) The incidence of lymph node metastasis in early gastric cancer according to the expanded criteria in comparison with the absolute criteria of the Japanese Gastric Cancer Association: a systematic review of the literature and meta-analysis. Gastrointest Endosc 87(2):338–347. https://doi.org/10.1016/j.gie.2017.09.025
18. DiBrito SR, Blair AB, Prasath V et al (2020) Total gastrectomy for CDH-1 mutation carriers: an institutional experience. J Surg Res 247:438–444. https://doi.org/10.1016/j.jss.2019.09.062
19. Corso G, Montagna G, Figueiredo J et al (2020) Hereditary gastric and breast cancer syndromes related to CDH1 germline mutation: a multidisciplinary clinical review. Cancers (Basel) 12(6):1598. https://doi.org/10.3390/cancers12061598
20. Forrester JD, Foster D, Ford JM et al (2022) Surgery for hereditary diffuse gastric cancer: long-term outcomes. Cancers (Basel) 14(3):728. https://doi.org/10.3390/cancers14030728

Prophylactic Total Gastrectomy: How Many?

16

Francesco Ferrara, Giulia Massari, Valentina Tagliaferri, and Giovanni Corso

Abstract

Individuals with *CDH1* germline mutations are at high risk of developing diffuse gastric cancer and prophylactic total gastrectomy represents the only life-saving treatment. Literature has reported a total of 224 surgical procedure in high-risk individuals, associated with germline *CDH1* pathogenic mutations. The majority were described in the USA, the Netherlands, and Canada. Gastric tumor was identified in almost 85.4% of cases after prophylactic surgery, and a high rate of "no cancer" at histopathology was identified in the USA. Considering the mutation type, most of alterations were nonmissense versus missense sub-types. In this chapter, we will describe the penetrance risks for gastric cancer in *CDH1* carriers and their implication for prophylactic gastrectomy.

16.1 Introduction

Prophylactic total gastrectomy (PTG) has been proposed by many authors for patients with asymptomatic *CDH1* mutation carriers, the genetic background for the development of hereditary diffuse gastric cancer (HDGC). Although the age at which this procedure can be done has not yet been precisely defined, it has been

F. Ferrara
Department of Surgery, San Carlo Borromeo Hospital, ASST Santi Paolo e Carlo, Milan, Italy

G. Massari · V. Tagliaferri
Division of Breast Surgery, European Institute of Oncology, IRCCS, Milan, Italy

G. Corso (✉)
Division of Breast Surgery, European Institute of Oncology, IRCCS, Milan, Italy

Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy
e-mail: giovanni.corso@ieo.it

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_16

proposed by many authors in the second decade of life, to eliminate the risk of developing lethal GC [1]. In HDGC caused by *CDH1* germline mutations, the risk of developing GC should be higher than 80% and the average age at diagnosis of GC is younger than 40 years of age, with a range from less than 20 to more than 70 years [2]. Since the risk of developing GC is high and the value of health surveillance for high-risk lesions is still limited, prophylactic gastrectomy is recommended for mutation carriers in these families [3].

16.2 Criteria for *CDH1* Genetic Test

Firstly, in 1999, the International Gastric Cancer Linkage Consortium (IGCLC) defined families with the HDGC syndrome associated with *CDH1* germline mutations as those fulfilling one of the following features: (a) two or more documented cases of diffuse gastric cancer (DGC) in first- or second-degree relatives, with at least one diagnosed before the age of 50; (b) three or more cases of documented DGC in first- or second-degree relatives, independent of the age of onset [4].

However, due to the increase in the *CDH1* germline mutation rate, those initial criteria have become insufficient. Recently, novel international guidelines for *CDH1* genetic screening have been published as follows:

Family Criteria: (a) ≥2 cases of GC in family regardless of age, with at least one DGC; (b) ≥1 case of DGC at any age and ≥ 1 case of LBC at age < 70 years in different family members; (c) ≥2 cases of LBC in family members <50 years of age.

Individual Criteria: (d) DGC at age <50 years; (e) DGC at any age in individuals of Māori ethnicity; (f) DGC at any age in individuals with a personal or family history (first-degree relative) of cleft lip or cleft palate; (g) history of DGC and LBC, both diagnosed at age <70 years; (h) bilateral LBC, diagnosed at age <70 years; (i) gastric in situ signet ring cells or pagetoid spread of signet ring cells in individuals <50 years of age [3]

16.3 Penetrance Risk

DGC is the main cancer phenotype associated unequivocally with the germline E-cadherin pathogenic mutations. To date, it is assessed that about 80–90% of GC appears as sporadic forms, 10–20% are within a familial setting; however, only 1–3% are related to documented germline causes. In accord with the latest literature-review, the majority of HDGC families segregate only for DGC, without association with other cancers' phenotypes [5]. Ninety-five families present a classic HDGC phenotype, accounting in about 66% of all screened pedigrees (unpublished data, personal archive). Penetrance risk for DGC development in germline *CDH1* mutation carriers is not "fixed", it appears variable depending on several factors: country of origin (high- vs. low-risk area for GC), mutation subtypes

Table 16.1 Estimated penetrance risks reported in literature in accord with the different tumor sub-type

		Criteria % (95% CI)			
Tumor phenotype	Gender	IGCLC	Overall	Familial	Unselected
Stomach	Male	70 (59–80)	42 (30–56)	64 (43–87)	37.2 (8.7–89.5)
	Female	56 (44–69)	33 (21–43)	47 (29–60)	24.7 (6.1–68.9)
Breast	Female	42 (23–68)	55 (39–68)	–	42.9 (33.4–53.9)
Prostate	Male	–	3.2 (1–9.4)	6.3 (1.6–23.9)	2.7 (0.8–8.7)
Colorectum	Male	–	7 (0–17)	–	–
	Female	–	4 (0–11)	–	–

(truncating vs. nontruncating), family history (positive vs. negative), adopted clinical criteria (stringent vs. extended).

Hansford and colleagues reported that in individuals meeting the IGCLC 2010 criteria and with *CDH1* germline mutation [6], the cumulative lifetime GC risk at 80 years of age was 70% (95% CI, 59–80%) for males and 56% (95% CI, 44–69%) for females [7]. More recently, Roberts and colleagues reported that in individuals with *CDH1* pathogenic variants identified by MultiGene Panel Testing (MGPT) who did not meet established clinical testing criteria, the CI of GC at age 80 years was significantly lower: 42% (95% CI, 30%–56%) for men and 33% (95% CI, 21%–43%) for women. Stratifying by number of reported cases of GCs per family, the estimated cumulative incidence of GC was 64% (95% CI, 43%–87%) for men and 47% (95% CI, 29%–60%) for women in families reporting three or more GCs and 27% (95% CI, 15%–41%) for men and 24% (95% CI, 12%–36%) for women reporting two or fewer GCs [8]. Moreover, in unselected GC patients with *CDH1* mutations, the cancer risk decreases further. Xicola et al. have estimated an overall cumulative risk of GC by age 80 around 37.2% for men and 24.7% for women [9]. It is interesting to note that the presence of a positive family history for GC, increases the GC risk in germline *CDH1* mutations carriers (Table 16.1).

16.4 Indication

Measures to contain the risk are PTG or gastric endoscopic surveillance. In HDGC with exclusive DGC manifestation, endoscopic surveillance seems insufficient to detect early gastric lesions associated to *CDH1* mutations, because tumor is often multifocal, tumor cells infiltrating the mucosa, with a normal surface epithelium, and each focus is very often less than 1 mm in greatest diameter [10]. However, yearly endoscopic surveillance is the only alternative to PTG, if patient refuses it. It is also recommended the eradication of *Helicobacter Pylori* if present.

The latest IGCLC guidelines recommend PTG in *CDH1* variant carriers from families with confirmed HDGC, irrespective of endoscopic findings. Surgery should be proposed between 20 and 30 years of age, but not recommended in elderly individuals (>70 years old), due to the increased perioperative risks [3].

In this context, although PTG is considered the unique life-saving option in germline *CDH1* pathogenic carriers, we must consider some important points: individuals with germline *CDH1* nontruncating mutations [11] and without a clear family history for GC seem associated with a lower penetrance of GC risk. This surgical procedure should be considered only in case of a clear HDGC phenotype, and with documented germline *CDH1* pathogenic variants. Individuals with variants of unknown significance alterations are not eligible for PTG [3], and some authors discourage PTG also in case of nontruncating mutation carriers in absence of a clear family history for GC.

DiBrito et al. [12] have reported a series of PTGs about ten patients with germline *CDH1* mutation and fulfilling the standard IGCLC clinical criteria [6]. The tumor identification rate in this small population was high (50%), but, when considering only cases actually treated with PTG, 38% of patients (without cancer before surgery) presented cancer in resected gastric specimens. In detail, they performed a total of ten PTGs; two patients presented GC in preoperative endoscopy, whereas eight individuals were "potentially" eligible for PTG because no cancer was detected by endoscopy before surgery. However, final pathological examination revealed cancer in five patients, including three of the eight with negative endoscopic findings. Rightly so, the authors discussed the benefits of PTG, stating that a very high rate of tumor identification after PTG (between 67% and 100%) is reported in the literature. Nevertheless, criticism has been leveled at the real benefits of PTG in cases that do not fulfill these criteria, particularly in the absence of significant family aggregation of GC.

The introduction of multigene panel testing for measuring hereditary cancer susceptibility has seen an increase in the number of *CDH1* mutations detected [13]. Although a rare event, such mutations have been discovered in patients who do not fulfill the clinical criteria established by the IGCLC. Rightly so, some authors have discussed the real benefits of PTG in incidental findings or in the absence of familial aggregations of GC [14].

HDGC is a complex and multifactorial cancer syndrome with inherited predisposition, showing different phenotypes, but with predominant aggregation of DGC and lobular breast cancer (LBC). The exact genetic mechanisms causing (or excluding) gastric-breast tumorigenesis are still unclear, as these pathways could overlap or totally diverge. Recent studies identified *CDH1* germline mutations also in patients with just LBC and without aggregation of DGC [15].

Using multigene panel testing, Hamilton et al. [13] retrospectively described several *CDH1* germline mutations (16 pathogenic and 41 variants of uncertain significance), only 26.3% of which associated to a family history of GC. PTG was performed on nearly 40% of the individuals carrying pathogenic variants and on none of those with variants of uncertain significance. It remains unclear on what basis the individuals who underwent PTG were chosen. The authors speculated that the risk of GC may be lower in individuals with pathogenic *CDH1* and no family history of GC than in those with a positive family history.

This study poses a new question: should PTG be always recommended to asymptomatic individuals who are positive to the *CDH1* test? Recent clinical

evidence has clearly demonstrated that a distinct HDGC genotype-phenotype exists; we can distinguish at least three different clinical settings for the HDGC syndrome: (a) the "classic" HDGC syndrome with aggregation of DGC and with or without history of LBC, (b) the "syndromic" HDGC with aggregation of only LBC and without DGC history (so-called HLBC) [16], and (c) incidental findings (asymptomatic individuals), with unclear family history and absent or insignificant aggregation of DGC and/or LBC.

For cases of "classic" HDGC syndrome, PTG remains the only life-saving option because of the high risk of developing DGC. However, for families with an HLBC pattern and for incidental findings, PTG should be considered with caution, because the association with DGC in *CDH1* germline mutant carriers is not conclusive, and no genetic studies are available that evaluate the real risk of developing DGC in these families, whose risk of developing LBC or other tumors might be higher. Based on these considerations, we point out that, at present, PTG is not recommended as standard practice and that there is no standard strategy for reducing the risk of GC in families that do not meet the established IGCLC clinical criteria.

16.5 Geographic Distribution

Information about the country of origin in the worldwide distribution of *CDH1* mutations is lacking in 55% of literature reports. About 45% of alterations have been described and detected in individuals from European origin, with lower percentages identified in Asian and American subjects (26.3% and 16.0%, respectively), as well as in Oceania (11.5%). The predominant mutation type varies across geographical regions. Deletions are more frequent in Europe (34%), splice-site in America (48%), missense in Asia (68%), and nonsense in Oceania (78%). The high prevalence of missense mutations in Asia is mainly attributed to Korean and Japanese populations [5].

In a recent systematic review, 224 surgical procedures classified as PTG, with an age range of 18–71 years, have been found in the literature. Most of PTGs have been performed in the USA [112 (50%)] followed by the Netherlands [40 (17.8%)], Canada [28 (12.5%)], Belgium [8 (3.6%)], Spain [8 (3.6%)], Denmark [7 (3.1%)], Portugal [6 (2.7%)], Austria [6 (2.7%)], Mexico [4 (1.8%)], Iran [2 (0.9%)], Australia [1 (0.4%)], Chile [1 (0.4%)], Germany [1 (0.4%)], and Italy [1 (0.4%)], respectively (Fig. 16.1) [17].

At histopathology findings, 32 (14.6%) cases showed "no cancer" after PTG, in 159 (72.6%) cases at least one focus of signet ring cell carcinoma (SRCC) was identified and in 28 (12.8%) cases an invasive tumor was identified. A higher rate of "no cancer" detections was reported in the USA and Belgium in comparison to the others, however considering the three countries with the highest number of reported PTGs (USA, Canada, and the Netherlands). Canada and the Netherlands reported a lower number of "no cancer" results in histopathology findings after PTG in comparison to the USA.

Fig. 16.1 Global geographic distribution of prophylactic total gastrectomies performed in the world. Most of these surgical procedures were reported in the USA (original figure)

So PTGs are primarily performed in Western countries, in particular in the USA, the Netherlands, and Canada. The higher frequency of "no cancer" detections in the USA compared to the other countries coincides with higher rates of *CDH1* germline alterations identified at the time of genetic screening in countries with a lower incidence of GC. However, since in high-risk areas for GC, the genetic screening for *CDH1* is rarely obtained, due to the high likelihood of obtaining a negative result maybe for environmental factors, it is possible that the geographic distribution of performed PTGs is heterogeneous and might reflect the frequency of *CDH1* test results [18].

In the USA, the lower rate of SRCC at PTG specimens may be associated with the absence of family history of GC, but in these subjects, there is no indication to perform a PTG because the risk of developing a *CDH1*-associated SRCC amongst asymptomatic individuals with a *CDH1* gene alteration, but with a negative family history of GC, is lower than in those with a relevant family history of GC. Moreover, the progression of foci of SRCC to aggressive DGC is not clear [19].

Most of PTGs have been performed in individuals carrying a *CDH1* nonmissense mutation (86.9% vs. 13.1% missense), so physicians are often reluctant to propose PTG in patients with *CDH1* missense mutations. HDGC missense mutation carriers are very difficult to manage since this type of mutation occurs in 30% of cases. Furthermore, most of the *CDH1* rule specifications in the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) variant curation guidelines are not recommended for use in missense changes, and a large proportion of variants remain unclassified. So, in these cases it is recommended to perform a complementary set of analyses and to date there are no international guidelines for the performance of PTG in patients with missense mutations of unknown significance [20].

16.6 Conclusion

CDH1 mutations are more frequently identified in countries with low incidence for GC, and the application of criteria for genetic screening is critical for a higher detection rate, as well as for the identification of mutations with proven clinical relevance. In the HDGC spectrum associated with *CDH1* germline pathogenic alterations, PTG remains the only life-saving option due to the high risk of developing a gastric carcinoma, and it should be strongly recommended. In other *CDH1*-associated conditions, this surgical approach is still a matter of debate, which should be discussed in a multidisciplinary forum, also involving the patient in the decision-making process.

Note

Parts of this chapter are based on the cited publications by Corso et al. (2022) [11], Corso et al. (2022) [12], and Corso (2020) [21].

References

1. Pedrazzani C, Corso G, Marrelli D et al (2007) E-cadherin and hereditary diffuse gastric cancer. Surgery 142(5):645–657. https://doi.org/10.1016/j.surg.2007.06.006
2. Kluijt I, Siemerink EJ, Ausems MG et al (2012) CDH1-related hereditary diffuse gastric cancer syndrome: clinical variations and implications for counseling. Int J Cancer 131(2):367–376. https://doi.org/10.1002/ijc.26398
3. Blair VR, McLeod M, Carneiro F et al (2020) Hereditary diffuse gastric cancer: updated clinical practice guidelines. Lancet Oncol 21(8):e386–e397. https://doi.org/10.1016/S1470-2045(20)30219-9
4. Caldas C, Carneiro F, Lynch HT et al (1999) Familial gastric cancer: overview and guidelines for management. J Med Genet 36(12):873–880. https://doi.org/10.1136/jmg.36.12.873
5. Corso G, Corso F, Ballerba F et al (2021) Geographical distribution of E-cadherin germline mutations in the context of diffuse gastric cancer: a systematic review. Cancers 13(6):1269. https://doi.org/10.3390/cancers13061269
6. van der Post RS, Vogelaar IP, Carneiro F et al (2015) Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. J Med Genet 52:361e374. https://doi.org/10.1136/jmedgenet-2015-103094
7. Hansford S, Kaurah P, Li-Chang H et al (2015) Hereditary diffuse gastric cancer syndrome: CDH1 mutations and beyond. JAMA Oncol 1(1):23–32. https://doi.org/10.1001/jamaoncol.2014.168
8. Roberts ME, Ranola JM, Marshall ML et al (2019) Comparison of *CDH1* penetrance estimates in clinically ascertained families vs families ascertained for multiple gastric cancers. JAMA Oncol 5(9):1325–1331. https://doi.org/10.1001/jamaoncol.2019.1208
9. Xicola RM, Li S, Rodriguez N, Reinecke P et al (2019) Clinical features and cancer risk in families with pathogenic CDH1 variants irrespective of clinical criteria. J Med Genet 56(12):838–843. https://doi.org/10.1136/jmedgenet-2019-105991
10. Corso G, Montagna G, Figueiredo J et al (2020) Hereditary gastric and breast cancer syndromes related to CDH1 germline mutation: a multidisciplinary clinical 232 review. Cancers 12(6):1598. https://doi.org/10.3390/cancers12061598
11. Corso G (2022) Pleiotropic cancer manifestations of germline CDH1 mutations: risks and management. J Surg Oncol. https://doi.org/10.1002/jso.26847

12. DiBrito SR, Blair AB, Prasath V et al (2020) Total gastrectomy for CDH-1 mutation carriers: an institutional experience. J Surg Res 247:438e444. https://doi.org/10.1016/j.jss.2019.09.062
13. Hamilton JG, Long JM, Brandt AC et al (2019) Patients' medical and psychosocial experiences after detection of a CDH1 variant with multigene panel testing. JCO Precis Oncol 3: PO.18.00300. https://doi.org/10.1200/PO.18.00300
14. Katona BW, Farengo Clark D, Domchek SM (2020) CDH1 on multigene panel testing: look before you leap. J Natl Cancer Inst 112:330e334. https://doi.org/10.1093/jnci/djz229
15. Corso G, Intra M, Trentin C et al (2016) CDH1 germline mutations and hereditary lobular breast cancer. Familial Cancer 15:215e219. https://doi.org/10.1007/s10689-016-9869-5
16. Corso G, Figueiredo J, La Vecchia C et al (2018) Hereditary lobular breast cancer with an emphasis on E-cadherin genetic defect. J Med Genet 55:431e441. https://doi.org/10.1136/jmedgenet-2018-105337
17. Corso G, Magnoni F, Nicastro V et al (2022) Global distribution of prophylactic total gastrectomy in E-cadherin (CDH1) mutations. Semin Oncol 49:130
18. Corso G, Marrelli D, Pascale V et al (2012) Frequency of CDH1 germline mutations in gastric carcinoma coming from high- and low-risk areas: metanalysis and systematic review of the literature. BMC Cancer 6(12):8. https://doi.org/10.1186/1471-2407-12-8
19. Stewart DR, Frone MN, Chanock SJ (2020) Stomaching multigene panel testing: what to do about CDH1? J Natl Cancer Inst 112(1):325–326. https://doi.org/10.1093/jnci/djz230
20. Lee K, Krempely K, Roberts ME et al (2018) Specifications of the ACMG/AMP variant 254 curation guidelines for the analysis of germline CDH1 sequence variants. Hum Mutat 39(11):1553–1568. https://doi.org/10.1002/humu.23650
21. Corso G (2020) Letter regarding: is prophylactic Total gastrectomy always indicated in CDH1 germline mutant carriers? J Surg Res 255:647–648. https://doi.org/10.1016/j.jss.2020.02.022

Hereditary Lobular Breast Cancer Syndrome: Role of Surgery

17

Francesca Magnoni, Antonia Girardi, and Paolo Veronesi

Abstract

Hereditary lobular breast cancer is a novel and rare inherited syndrome, and it represents a multifaceted and fascinating clinical entity. In recent years, it has been arousing intense scientific research, actively involving clinicians and researchers specializing in different fields, in order to clearly and precisely outline its specific management, which is currently particularly nuanced and controversial. In this sense, prophylactic and therapeutic surgery plays a significant role in the management of affected women: diversified clinical, genetic, and individual considerations should be contemplated in a multidisciplinary fashion, adhering to available international scientific evidence and guidelines.

The chapter offers a surgical perspective on current knowledge of this issue, also reporting a technical and specialized study of preventive mastectomy.

17.1 Introduction

Breast cancer (BC) is the foremost cause of cancer and cancer death in women, with about two million cases and 700,000 deaths registered worldwide [1]. Lobular breast carcinoma (LBC) is the second most common "special" morphological subtype of BC and comprises up approximately 10–15% of all breast cancer cases [2]. It is typically characterized by small, noncohesive epithelial cells dispersed individually

F. Magnoni (✉) · A. Girardi
Division of Breast Surgery, IEO European Institute of Oncology, IRCCS, Milan, Italy
e-mail: francesca.magnoni@ieo.it

P. Veronesi
Division of Breast Surgery, IEO European Institute of Oncology, IRCCS, Milan, Italy

Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_17

in a single-file linear pattern in a fibrous stroma with loss of expression of the cell adhesion molecule E-cadherin [3], resulting in cellular unregulated growth, metastases, and worse prognosis [4].

The majority of LBCs are hormone receptor positive with low proliferation index [5–7]. It represents a nonaggressive prognostic phenotype, with low histological grade and a generally favorable response to endocrine therapy [8]. However, when it is correlated to the E-cadherin dysfunction, it shows a cellular discohesive pattern and a loss in tissue basic structure: the presence of a *CDH1* mutation causes deregulation of E-cadherin function, resulting in decreased cell-cell adhesion and increased cell proliferation (so-called lobular hyperplasia) [9] . The E-cadherin dysfunction, master regulator of the lobular phenotype [10], results in a marked predisposition to wide metastatic spread, synonyms of considerable invasiveness, with worse impact on outcome compared with nonspecial type BC [3].

The surgical management of this fascinating and complex type of malignancy, in the presence of germline E-cadherin mutations, at present is extensively studied: it always requires careful and specialized clinical and genetic counseling, adhering to patient's wishes.

17.2 Hereditary Lobular Breast Cancer

Familial and genetic factors are recognized risk factors for BC, particularly for early-onset cancers and familial clusters [11]. Additionally, enhanced diagnostic tools in genetic assessment are implementing the ability to identify high-risk women with elevated risk for breast cancer through genetic testing [12], involving the consideration that several risk management options are available to support women at higher than average risk of BC.

BRCA2 mutations are associated with both ductal and LBC, whereas deregulation of *CDH1* is uniquely associated with LBC. One of the characteristic features of invasive LBC is the loss of E-cadherin expression and function in about 90% of cases [13].

A germline mutation of *CDH1* is responsible for hereditary diffuse gastric cancer (HDGC), an autosomal-dominant inherited syndrome [14].

Hereditary lobular breast cancer (HLBC) is an infrequent inherited disease associated with *CDH1* germline mutation, without apparent correlation with the HDGC [9]. Indeed, the presence of pathogenic *CDH1* mutations in either an isolated individual with LBC, or a family with one or more LBC cases in first-degree or second-degree relatives is correlated with HLBC, but without a family history of DGC in either situation [15]. Moreover, if LBC is a precursor manifestation of the HDGC syndrome or an isolated and independent cancer inherited predisposition is still uncertain. Several genetic studies have recognized novel germline *CDH1* mutations in LBCs correlated with the HDGC syndrome [16, 17], indeed LBC is associated with HDGC and E-cadherin constitutional mutations have been reported in both gastric and breast cancers [18].

The cumulative incidence of cancer risk for BC in the HLBC setting is unknown [19]. Likely, the risk to develop diffuse gastric cancer (DGC) is not the same in all families carrying pathogenic *CDH1* mutations, due to the role of environmental factors which could play a synergic role in gastric cancer (GC) development [20].

Thus, women with pathogenic *CDH1* variants present an elevated lifetime risk of invasive LBC, in addition to an increased risk of GC [21]: female *CDH1* mutation carriers meeting the International Gastric Cancer Linkage Consortium (IGCLC) 2010 criteria [22], have in fact a risk of BC of 42% (95% CI, 23–68%), mostly being LBC [23–25]. LBC with pathogenic *CDH1* mutation is associated with two different clinical phenotypes, "mixed" HDGC and HLBC [19]. In HLBC syndrome, BC can occur in the absence of a family history of DGC.

In HLBC syndrome, LBC manifestation emerges sooner than in "mixed" HDGC.

Preventive strategies (surveillance or prophylactic surgery) should be taken into account, based on these different phenotypes.

In a recent study [19], some interesting discussion points emerged. The overall *CDH1* mutation frequency is higher in the HLBC family group (70.5%), in comparison to the "mixed" HDGC group. Authors demonstrated that LBC patients with *CDH1* mutations more frequently present an "isolated" BC phenotype without DGC manifestation. Furthermore, they underlined an earlier onset of LBC in families with HLBC compared to those with "mixed" HDGC, in which GC occurs earlier. These data suggest that HLBC is a possible independent syndrome, in which LBC predominates.

17.3 *CDH1* Gene Mutation Carriers' Management

Clinical management of heritable *CDH1* gene mutation carriers is challenging and object of considerable scientific research [26]. The IGCLC clinical criteria stated as mandatory for *CDH1* genetic screening, in the presence of personal or family history of HDGC and LBC, one diagnosed <50 years [9, 22]. Testing has been also considered an option for families with bilateral LBC or family history of two or more cases of LBC <50 years [9, 22]. To date, IGCLC endorsed that E-cadherin genetic screening associated with LBC can be reconsidered in both LBC in the setting of the HDGC syndrome, and HLBC not associated with GCs [9].

The presence of *CDH1* germline mutations identified in cases of LBC not associated with the classical HDGC syndrome have required the need to define specific new criteria for recognition of patients at risk of HLBC. Indeed, bilateral LBC with or without family history of LBC, with age at onset <50 years, and unilateral LBC with family history of LBC, with age at onset <45 years have been validated as affective criteria by a novel working group dedicated to clinical and genetic management of HLBC [26–28].

Breast surveillance in *CDH1* mutation carriers lacks a unique and shared protocol due to the limited cases of identified *CDH1* germline mutations reported in literature and not the substantial data concerning BC risk in these subjects [9]. In mutated *CDH1* women, timely radiological breast monitoring is however recommended, due

to the high risk of LBC developing [17], even if there are no international guidelines on breast radiological surveillance in these individuals, unlike for ascertained *BRCA1/2* genetic mutations' carriers [26].

Corso and colleagues recommended the use of annual breast MRI followed by mammography and ultrasound at 6-months interval, as defined for *BRCA1/2* carriers [29]. In addition, updated clinical practice guidelines recommended starting surveillance for HDGC and HLBC at 30 years of age, with yearly MRI from 30 to 50 years of age and potentially for longer. Such recommendations also pointed out the uncertain benefit of adding mammography in young women and the role of supplemental screening ultrasound in dense breasts when MRI is not feasible [15].

17.4 Prophylactic Surgery

The risk management of *CDH1* mutation carriers should firstly distinguish between never affected individuals and patients diagnosed with BC [29].

Recent ASCO 2020 guidelines revealed a de-escalation and personalization in breast surgery recommendations in LBC patients, indeed they stated with a moderate recommendation that both *BRCA* and moderate-penetrance gene mutations' carriers could be eligible for breast-conserving therapy, when this is clinically proper [30, 31].

No conventionally accepted protocol for prophylactic surgery has been defined, given the low number of *CDH1* germline mutations identified and the few data on the risk of BC development in *CDH1* mutant carriers [9].

Data are insufficient to recommend contralateral surgery to reduce the risk of BC for affected *CDH1* mutation carriers [9] and prophylactic surgery to healthy individuals with *CDH1* mutation, even if also family history, ability to undergo high-risk screening procedures, and patient preference are main factors to take in account in the decision process [26, 32].

For carriers of the *CDH1* mutation, the literature suggests that prophylactic mastectomy (PM) could be discussed in selected cases, after a genetic counseling as well as after a multidisciplinary discussion and in accordance with women's preferences [9, 26, 32].

As there is currently no firm evidence in favor of PM, the possibility of risk-reducing surgery should be discussed in relation to the potential presence of LBC in the personal medical history of *CDH1* mutation carriers. As we recently have reported [26], a specific plan on surgical management for *CDH1* carriers has been recently defined: information on risk-reducing surgery should be provided to *CDH1* positive patients with diagnosis of LBC, who have a clinical indication for mastectomy or already had a mastectomy as part of their cancer treatment [29, 33]. Prophylactic surgery should be offered as well to individuals with a positive family history for LBC and a well-documented *CDH1* pathogenic alteration in a first-degree relative [29, 33].

17.5 Technical and Clinical Implications of Prophylactic Mastectomy

Well-known and validated is the role of "conservative mastectomy" [34], meant as skin and nipple-sparing. The first approach combines the total excision of the mammary gland, sparing the skin, the second, preserves both the skin and the nipple-areola complex (NAC).

The intent of preventive mastectomy is to obtain maximum risk reduction, removing enough breast tissue for optimal risk reduction, as well as to provide the best cosmetic outcome thanks to reconstructive surgery. Given the greater attention to women's body image and sexual and psychological well-being, skin and nipple-sparing mastectomy with immediate reconstruction in both therapeutic and prophylactic settings have been increasing significantly [35].

Indeed, scientific evidence reported that, as defined for *BRCA* mutation carriers [36], the gold standard for *CDH1* mutation carriers appears to be represented by nipple-sparing mastectomy (NSM) with immediate reconstruction, which, thanks to the preservation of the skin envelope and the nipple-areola complex, can optimize the oncological and esthetic results [29] with excellent oncological safety and low complications rate [37–40]. NSM presents, albeit in a low percentage, a risk of complications, which should be discussed with *CDH1*-mutated women in the multidisciplinary evaluation of the possible risk reduction options.

The possible oncological failure after prophylactic mastectomy in literature is described as a residual risk of about 5%, to be related to the possible presence of residual glandular tissue or ectopic breast tissue [41]. Review by Muller et al. highlights the extremely low risk of cancer development following breast risk-reducing surgery. Specifically, from the 3716 cases of prophylactic NSM, only nine cases (0.2%) of BC exterior NAC and one case (0.004%) within the NAC were reported [40].

Nipple-sparing mastectomy is catheterized by a surgical morbidity: Jakub et al. described an overall complication rate of 15–20% [42]. In a recent large meta-analysis of NSMs, an overall complication rate of 22% and a nipple necrosis rate of 5.9% were reported [37], Galimberti et al. confirmed the oncological safety of the NAC preservation, describing a nipple necrosis rate of 3.5% and 2.2%, in invasive and in situ cancers respectively, slightly lower than reported elsewhere [39].

In relation to quality of life, literature reports a high overall patient satisfaction rate after bilateral prophylactic mastectomy, maybe due to patients experiencing less anxiety over BC after the procedure: most patients (70%) are satisfied with the surgery, the majority (65%) of women maintain a positive body image following prophylactic mastectomy and 95% of women had no regret following bilateral prophylactic mastectomy [43]. Moreover, this procedure appears generally well accepted thanks to the NAC conservation and consequent esthetic outcome improvement [38, 44].

Nipple-sparing mastectomy requires a double-time approach, the gland dissection and the reconstructive surgery, preserving NAC. A correct technical method requires maximum attention to completely and precisely remove the mammary gland, with

respect of all its anatomical limits, saving skin envelope and the inframammary folds to achieve a better immediate breast reconstruction. The dissection is very close to the dermis but maintaining a thin portion of subcutaneous fat to preserve the sub-dermal vascularization. Breast surgeons must also pay high attention to accurately remove the glandular tissue underneath the nipple, but without however damaging its vitality [9, 45].

The upper outer linear skin incision is the most used, but surgical approach can be personalized in relation to several clinical peculiarities: indeed, different skin incisions for NSM have been proposed in literature. According to the breast size, areola size, different types of breast ptosis, further "non-standard" surgical accesses were documented. Hemi-periareolar, round block, vertical pattern, and wise pattern skin incisions were described by Corso and Colleagues as the heterogeneous, safe, and effectual evolutions of conventional NSM [46].

Besides, the development of technology in these recent years has led to important achievements, as well in surgery. In particular, robotic technique has been applied also in prophylactic breast surgery and it has been successfully tested in terms of technical accuracy, feasibility, satisfaction, and tolerance rate for women. The advantage of this minimally invasive technique consists in its capability to perform a complete NSM through a small extra-mammary skin access. Compared to the conventional standard NSM, it offers a good esthetic outcome, with the same technical effectiveness [47]. A randomized single center clinical trial comparing open NSM and robotic NSM has been recently closed,reporting at a median follow-up of 28.6 months no local events, low complications rate, and high postoperative quality of life [48].

17.6 Conclusions

HLBC represents an heterogenous and rare inherited predisposition associated with *CDH1* germline mutation, requiring accurate and expert multimodal approach. In this context of not straightforward clinical management [49], an international multidisciplinary workgroup of experts on HLBC revised key issues in this regard [29].

Selected *CDH1* mutant carriers could discuss prophylactic surgery in a multidisciplinary counseling and in highly specialized cancer centers. Indeed, prophylactic mastectomy should be considered in *CDH1* carriers with a strong aggregation for LBC, adhering to the established clinical criteria. Thus, contralateral prophylactic mastectomy can be a choice for patients with diagnosis of LBC, also following previous ipsilateral breast surgery [29].

Moreover, bilateral prophylactic mastectomy can be suggested in asymptomatic individuals with family history of LBC and well-documented *CDH1* pathogenic alterations in a first-degree relative. Clinical surveillance is still considered the best approach in case of *CDH1* mutations of unknown significance, (so called VUS). In asymptomatic *CDH1* carriers who do not fulfill the clinical criteria, BC surveillance is preferred [29].

References

1. Ferlay J, Colombet M, Soerjomataram I et al (2021) Cancer statistics for the year 2020: an overview. Int J Cancer 149:778–789. https://doi.org/10.1002/ijc.33588
2. Li CI, Anderson BO, Daling JR et al (2003) Trends in incidence rates of invasive lobular and ductal breast carcinoma. JAMA 289(11):1421–1424. https://doi.org/10.1001/jama.289.11.1421
3. Mouabbi JA, Hassan A, Lim B et al (2022) Invasive lobular carcinoma: an understudied emergent subtype of breast cancer. Breast Cancer Res Treat 149(4):778–789. https://doi.org/10.1007/s10549-022-06572-w
4. Ferlicot S, Vincent-Salomon A, Médioni J et al (2004) Wide metastatic spreading in infiltrating lobular carcinoma of the breast. Eur J Cancer 40(3):336–341. https://doi.org/10.1016/j.ejca.2003.08.007
5. Iorfida M, Maiorano E, Orvieto E et al (2012) Invasive lobular breast cancer: subtypes and outcome. Breast Cancer Res Treat 133(2):713–723. https://doi.org/10.1007/s10549-012-2002-z
6. Desmedt C, Zoppoli G, Gundem G et al (2016) Genomic characterization of primary invasive lobular breast cancer. J Clin Oncol 34(16):1872–1881. https://doi.org/10.1200/JCO.2015.64.0334
7. Richard F, Majjaj S, Venet D (2020) Characterization of stromal tumor-infiltrating lymphocytes and genomic alterations in metastatic lobular breast cancer. Clin Cancer Res 26(23):6254–6265. https://doi.org/10.1158/1078-0432.CCR-20-2268
8. Reed AEM, Kutasovic JR, Lakhani SR et al (2015) Invasive lobular carcinoma of the breast: morphology, biomarkers and 'omics. Breast Cancer Res 17:12. https://doi.org/10.1186/s13058-015-0519-x
9. Corso G, Figueiredo J, La Vecchia C et al (2018) Hereditary lobular breast cancer with an emphasis on E-cadherin genetic defect. J Med Genet 55(7):431–441. https://doi.org/10.1136/jmedgenet-2018-105337
10. McCart Reed AE, Kutasovic JR, Lakhani SR et al (2015) Invasive lobular carcinoma of the breast: morphology, biomarkers and 'omics. Breast Cancer Res 17(1):12. https://doi.org/10.1186/s13058-015-0519-x
11. Loibl S, Poortmans P, Morrow M et al (2021) Breast cancer. Lancet 397(10286):1750–1769. https://doi.org/10.1016/S0140-6736(20)32381-3
12. Tasleem JP, Wills CE, Yee LD et al (2017) Decision making for breast cancer prevention among women at elevated risk. Rev Breast Cancer Res 19(1):34. https://doi.org/10.1186/s13058-017-0826-5
13. Corso G, Figueiredo J, De Angelis SP et al (2020) E-cadherin deregulation in breast cancer. J Cell Mol Med 24(11):5930–5936. https://doi.org/10.1111/jcmm.15140
14. Caldas C, Carneiro F, Lynch HT et al (1999) Familial gastric cancer: overview and guidelines for management. J Med Genet 36(12):873–880
15. Blair VR, McLeod M, Carneiro F et al (2020) Hereditary diffuse gastric cancer: updated clinical practice guidelines. Lancet Oncol 21(8):e386–e397. https://doi.org/10.1016/S1470-2045(20)30219-9
16. Keller G, Vogelsang H, Becker I et al (1999) Diffuse type gastric and lobular breast carcinoma in a familial gastric cancer patient with an E-cadherin germline mutation. Am J Pathol 155:337–342. https://doi.org/10.1016/S0002-9440(10)65129-2
17. Corso G, Intra M, Trentin C et al (2016) CDH1 germline mutations and hereditary lobular breast cancer. Familial Cancer 15(2):215–219. https://doi.org/10.1007/s10689-016-9869-5
18. Corso G, Figueiredo J, Biffi R et al (2014) E-cadherin germline mutation carriers: clinical management and genetic implications. Cancer Metastasis Rev 33(4):1081–1094. https://doi.org/10.1007/s10555-014-9528-y
19. Girardi A, Magnoni F, Vicini E et al (2022) CDH1 germline mutations in families with hereditary lobular breast cancer. Eur J Cancer Prev 31(3):274–278. https://doi.org/10.1097/CEJ.0000000000000688

20. Corso G, Marrelli D, Pascale V et al (2012) Frequency of CDH1 germline mutations in gastric carcinoma coming from high- and low-risk areas: meta-analysis and systematic review of the literature. BMC Cancer 12:8. https://doi.org/10.1186/1471-2407-12-8
21. Gamble LA, Heller T, Davis JL (2021) Hereditary diffuse gastric cancer syndrome and the role of CDH1: a review [published online ahead of print, 2021 Jan 6]. JAMA Surg 156:387. https://doi.org/10.1001/jamasurg.2020.6155
22. van der Post RS, Vogelaar IP, Carneiro F et al (2015) Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. J Med Genet 52(6): 361–374. https://doi.org/10.1136/jmedgenet-2015-103094
23. Hansford S, Kaurah P, Li-Chang H et al (2015) Hereditary diffuse gastric cancer syndrome: CDH1 mutations and beyond. JAMA Oncol 1(1):23–32. https://doi.org/10.1001/jamaoncol.2014.168
24. Pharoah PD, Guilford P, Caldas C et al (2001) Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. Gastroenterology 121(6):1348–1353. https://doi.org/10.1053/gast.2001.29611
25. Kaurah P, MacMillan A, Boyd N et al (2007) Founder and recurrent CDH1 mutations in families with hereditary diffuse gastric cancer. JAMA 297(21):2360–2372. https://doi.org/10.1001/jama.297.21.2360
26. Corso G, Magnoni F, Trovato C et al (2022) Hereditary gastric cancer: a new syndrome. In: de Manzoni G, Roviello F (eds) Gastric cancer: the 25-year R-evolution. Updates in surgery. Springer, Cham. https://doi.org/10.1007/978-3-030-73158-8_5
27. Corso G, Bonanni B (2020) E-cadherin (CDH1 gene) germline mutations in gastric cancer: evolutions and innovations. Cancers (Basel) 12(10):2920. https://doi.org/10.3390/cancers12102920
28. Hansford S, Kaurah P, Li-Chang H et al (2015) (2015) Hereditary diffuse gastric cancer syndrome: CDH1 mutations and beyond. JAMA Oncol 1(1):23–32. https://doi.org/10.1001/jamaoncol.2014.168
29. Corso G, Montagna G, Figueiredo J et al (2020) Hereditary gastric and breast cancer syndromes related to CDH1 germline mutation: a multidisciplinary clinical review. Cancers (Basel) 12(6): 1598. https://doi.org/10.3390/cancers12061598
30. Tung NM, Boughey JC, Pierce LJ et al (2020) Management of hereditary breast cancer: American Society of Clinical Oncology, American Society for Radiation Oncology, and Society of Surgical Oncology Guideline. J Clin Oncol 38(18):2080–2106. https://doi.org/10.1200/JCO.20.00299
31. Corso G, Magnoni F (2021) Hereditary breast cancer: translation into clinical practice of recent American Society of Clinical Oncology, American Society of Radiation Oncology, and Society of Surgical Oncology recommendations. Eur J Cancer Prev 30(4):311–314. https://doi.org/10.1097/CEJ.0000000000000624
32. Jakub J, Peled AW, Gray RJ, Greenup RA et al (2018) Oncologic safety of prophylactic nipple-sparing mastectomy in a population with BRCA mutations: a multi-institutional study. JAMA Surg 153(2):123–129. https://doi.org/10.1001/jamasurg.2017.3422
33. Valachis A, Nearchou AD, Lind P (2014) Surgical management of breast cancer in BRCA-mutation carriers: a systematic review and meta-analysis. Breast Cancer Res Treat 144(3): 443–455. https://doi.org/10.1007/s10549-014-2890-1
34. Veronesi U, Stafyla V, Luini A, Veronesi P (2012) Breast cancer: from “maximum tolerable” to “minimum effective” treatment. Front Oncol 2:125. https://doi.org/10.3389/fonc.2012.00125
35. Magnoni F, Alessandrini S, Alberti L et al (2021) Breast cancer surgery: new issues. Curr Oncol 28(5):4053–4066. https://doi.org/10.3390/curroncol28050344
36. Rocco N, Montagna G, Criscitiello C et al (2021) Nipple-sparing mastectomy as a risk-reducing procedure for BRCA-mutated patients. Genes (Basel) 12(2):253. https://doi.org/10.3390/genes12020253

37. Headon HA, Kasem A, Mokbel K (2016) The oncological safety of nipple-sparing mastectomy: a systematic review of the literature with a pooled analysis of 12,358 procedures. Arch Plast Surg 43(4):328–338. https://doi.org/10.5999/aps.2016.43.4.328
38. Galimberti V, Vicini E, Corso G et al (2017) Nipple-sparing and skin-sparing mastectomy: review of aims, oncological safety and contraindications. Breast 34(Suppl 1):S82–S84. https://doi.org/10.1016/j.breast.2017.06.034
39. Galimberti V, Morigi C, Bagnardi V et al (2018) Oncological outcomes of nipple-sparing mastectomy: a single-center experience of 1989 patients. Ann Surg Oncol 25(13):3849–3857. https://doi.org/10.1245/s10434-018-6759-0
40. Muller T, Baratte A, Bruant-Rodier C et al (2018) Oncological safety of nipple-sparing prophylactic mastectomy: a review of the literature on 3716 cases. Ann Chir Plast Esthet 63(3):e6–e13. https://doi.org/10.1016/j.anplas.2017.09.005
41. Alaofi RK, Nassif MO, Al-Hajeili MR (2018) Prophylactic mastectomy for the prevention of breast cancer: review of the literature. Avicenna J Med 8:67–77. https://doi.org/10.4103/ajm.AJM_21_18
42. Jakub JW, Peled AW, Gray RJ et al (2018) Oncologic safety of prophylactic nipple-sparing mastectomy in a population with BRCA mutations. JAMA Surg 153(2):123–129. https://doi.org/10.1001/jamasurg.2017.3422
43. Razdan SN, Patel V, Jewell S et al (2016) Quality of life among patients after bilateral prophylactic mastectomy: a systematic review of patient reported outcomes. Qual Life Res 25(6):1409–1421. https://doi.org/10.1007/s11136-015-1181-6
44. van Verschuer VM, Mureau MA, Gopie JP et al (2016) Patient satisfaction and nipple-areola sensitivity after bilateral prophylactic mastectomy and immediate implant breast reconstruction in a high breast cancer risk population: nipple-sparing mastectomy versus skin-sparing mastectomy. Ann Plast Surg 77(2):145–152. https://doi.org/10.1097/SAP.0000000000000366
45. Franceschini G, Masetti R (2019) What the surgeons should know about the bilateral prophylactic mastectomy in BRCA mutation carriers. Eur J Breast Health 15(2):135–136. https://doi.org/10.5152/ejbh.2019.4651
46. Corso G, De Lorenzi F, Vicini E et al (2018) Nipple-sparing mastectomy with different approaches: surgical incisions, complications, and cosmetic results. Preliminary results of 100 consecutive patients at a single center. J Plastic Reconstruct Aesthetic Surg 71(12):1751–1760. https://doi.org/10.1016/j.bjps.2018.07.022
47. Toesca A, Invento A, Massari G et al (2019) Update on the feasibility and progress on robotic breast surgery. Ann Surg Oncol 26(10):3046–3051. https://doi.org/10.1245/s10434-019-07590-7
48. Toesca A, Sangalli C, Maisonneuve P (2021) A randomized trial of robotic mastectomy versus open surgery in women with breast cancer or BRCA mutation. Ann Surg. https://doi.org/10.1097/SLA.0000000000004969
49. Katona BW, Clark DF, Domchek SM (2020) CDH1 on multigene panel testing: look before you leap. J Natl Cancer Inst 112(4):330–334. https://doi.org/10.1093/jnci/djz229

Breast Reconstruction

18

Francesca De Lorenzi, Francesco Borelli, and Mario Alessandri-Bonetti

Abstract

Postmastectomy reconstruction is nowadays an integral part of breast cancer treatment, often performed in the immediate setting.

Among women with hereditary gastric and breast cancer syndromes, three different scenarios can be identified. Firstly, healthy women seeking for prophylactic mastectomies. Secondly, cancer patients requiring mastectomy at the tumor site and simultaneous risk-reducing mastectomy of the healthy breast. Thirdly, cancer patients who have been treated for primary cancer requiring risk-reducing mastectomies in a further stage. In this chapter, we present a schematic guide for reconstruction for each subpopulation of subjects and their peculiarities.

Postmastectomy reconstruction is nowadays an integral part of breast cancer treatment and care, and in the vast majority of cases it is performed in the immediate setting. Breast reconstruction is a women's right with positive psychological effects, able to preserve body integrity and femininity. It requires a close collaboration between general and plastic surgeons if a double team approach is used. However, the rates of reconstruction are largely varying according to different countries and single institutions [1, 2] and among women themselves [3, 4]. In fact, younger

F. De Lorenzi (✉)
Department of Plastic and Reconstructive Surgery, IEO – European Institute of Oncology, IRCCS, Milan, Italy
e-mail: francesca.delorenzi@ieo.it

F. Borelli
Department of Reconstructive and Aesthetic Plastic Surgery, University of Milan, I.R.C.C.S. Istituto Galeazzi, Milan, Italy

M. Alessandri-Bonetti
School of Reconstructive and Aesthetic Plastic Surgery, University of Milan, Milano, Italy

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_18

women and those without a previous diagnosis of breast cancer are more likely to have breast reconstruction than older women or those with a previous diagnosis of cancer large (D cup or larger) ptotic breast or those with multiple previous scars.

Breast reconstruction is personalized and tailored to each patient, taking in consideration patient anatomy and comorbidities, cancer staging, and oncologic rules if present and even patient desires. Patient expectations are very high and women are seeking for outstanding cosmetic results especially in case of prophylactic surgery; therefore, preoperative counseling is mandatory, providing information about the type of reconstruction and the expected results.

Among women with hereditary gastric and breast cancer syndromes, three different scenarios can be identified. Firstly, healthy women seeking for prophylactic mastectomies. Secondly, cancer patients requiring mastectomy at the tumor site and simultaneous risk-reducing mastectomy of the healthy breast. Thirdly, cancer patients who have been treated for primary cancer requiring risk-reducing mastectomies in a further stage. In this chapter, we present a schematic guide for reconstruction for each subpopulation of subjects and their peculiarities.

18.1 Prophylactic Mastectomies in Healthy Women

Among women with inherited predisposition of gastric and breast carcinoma, several strategies can be offered to reduce the risk, including surveillance and lifestyle, chemoprevention, and risk-reducing surgery.

In case of healthy breasts and risk-reducing surgery, a conservative mastectomy is usually performed with "esthetic" surgical incisions. Nipple and areola complex can be preserved. Surgical incisions may be hidden in natural folds of the healthy breast (i.e., inframammary fold, axillary region) or just around the areola, resulting in very natural breasts (Figs. 18.1 and 18.2).

However, although the goal of prophylactic mastectomy is to achieve maximum risk reduction as well as reducing general distress and anxiety related to cancer, it

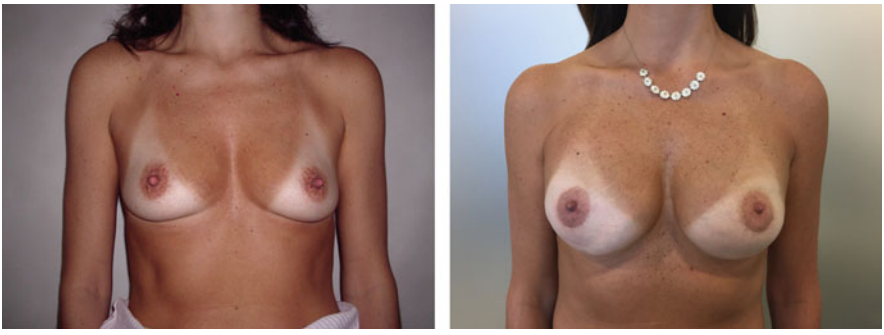


Fig. 18.1 Preoperative and postoperative results after bilateral risk-reducing mastectomy and direct-to-implant reconstruction through a periareolar skin incision

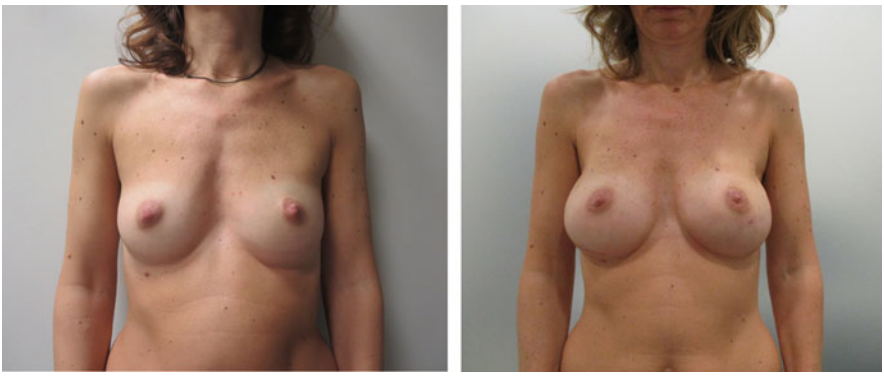


Fig. 18.2 Preoperative and postoperative results after bilateral risk-reducing mastectomy and direct-to-implant reconstruction with scars located at the level of the inframammary fold

represents a major surgery, it results in irreversible loss of breast sensitivity, it can be associated to minor and/or major complications resulting in unsatisfactory cosmetic outcomes. Moreover, the risk of local cancer recurrences exists. For these reasons, preoperative counseling is very important in unaffected CDH1 carriers, underling pros and cons of risk-reducing surgery tailored to every single woman. If women with small breasts may benefit of breast augmentation and improve their body contour, those with large (D cup or larger) and ptotic breasts or those with multiple previous scars are more prone to the onset of postoperative complications and anesthetic results. They are not ideal candidates for nipple-areola-sparing mastectomy and sometimes are better candidate to skin-sparing mastectomies [5, 6]. However, in the last decades, carefully designed reduction patterns have been successfully applied to retain a natural skin envelope (skin-reducing mastectomies) and to even maintain the areola and the nipple [7]. Also, in case of very large breast, the nipple and areola complex can be grafted on the reconstructed mound.

Finally, in unaffected CDH1 carriers, psychological counseling and support are very important to improve coping skills and the decision-making process among different risk-reducing strategies and several reconstructive options. The approach is personalized and takes in consideration the patient's personality, age, lifestyle, psychological well-being, social support, and self-efficacy.

Among healthy women requiring risk-reducing surgery, immediate reconstruction with definitive silicone gel implants is the most popular method of reconstruction [8]. Over the last decades, we observed an evolution of materials able to achieve really outstanding cosmetic outcomes. It is a simple procedure, for those women who do not like having significantly long surgeries with associated donor-site morbidity. It allows risk reduction and reconstruction in a single procedure. Even the knowledge of breast implant-associated large-cell lymphoma (BIA-ALCL) did not change the way of reconstruction, eventually moving to different implant surfaces [9, 10].

On the contrary, a minority of women prefer using autologous tissues, if applicable, because of the similarity to natural breast, stability of the long-term result, and anxiety about using foreign material/implants. Autologous reconstruction encompasses a broad range of procedures incorporating the patient's own tissues to recreate the breast mound (pedicled and free flaps, fat grafting).

In case of bilateral reconstructions as required after risk-reducing surgery, the differences in the long-term with both options are not significant [11]. In fact, unilateral implant reconstruction generally fails to fully "match" the contralateral breast without a bra and over time, this asymmetry actually worsen as the contralateral natural breast becomes more ptotic and changes in size with patient weight gain or loss. On the contrary, bilateral reconstructions with implants may be really satisfactory especially in case of small to medium breasts when nipple and areola are preserved or if reducing patterns in large breasts are successfully used.

18.1.1 Submuscular Implant-Based Reconstructions

Subpectoral implant reconstruction was first described in 1981 and it has been considered the standard of care for several decades. Since the beginning, the surgical technique has been modified with the introduction of more conservative approaches for mastectomy and newer generation implants.

In total submuscular approach, the implant is placed under the pectoralis muscle and/or serratus muscle and/or serratus fascia. In this way, the implant is covered and protected by a highly vascularized tissue. For this reason, the submuscular approach has traditionally been perceived to be the "safest" with regard to postoperative complications such as wound dehiscence and mastectomy flap necrosis, infection, and implant loss. Moreover, a total submuscular pocket allows a complete separation of the implant space from the axillary space, being protective in case of fluid collection from the axilla.

The subpectoral technique is considered the first choice in patients with comorbidities such as uncontrolled diabetes, obesity, smokers, as well as those with a history of previous breast surgery and multiple scars (Fig. 18.3). These patients may benefit from implant placement either completely or partially under the pectoralis muscle to provide an additional vascularized layer between mastectomy flaps and the implant. Also, breast size and ptosis are critical points to consider. Increasing breast size and ptosis will result in a higher risk of impaired skin perfusion of the mastectomy flaps, therefore a muscular coverage may be desirable.

In 2011, Salgarello et al. [12] described a subpectoral/subfascial implant pocket that represents an evolution of the classical submuscular pocket. In the upper pole, the implant is covered by the pectoralis major muscle that is elevated in continuity with the superficial pectoralis fascia up to the inframammary fold. Therefore, in the inferior pole, the implant is covered by the fascial system. This approach allows a larger pocket able to provide lower pole fullness and projection and the immediate insertion of the definitive implant.

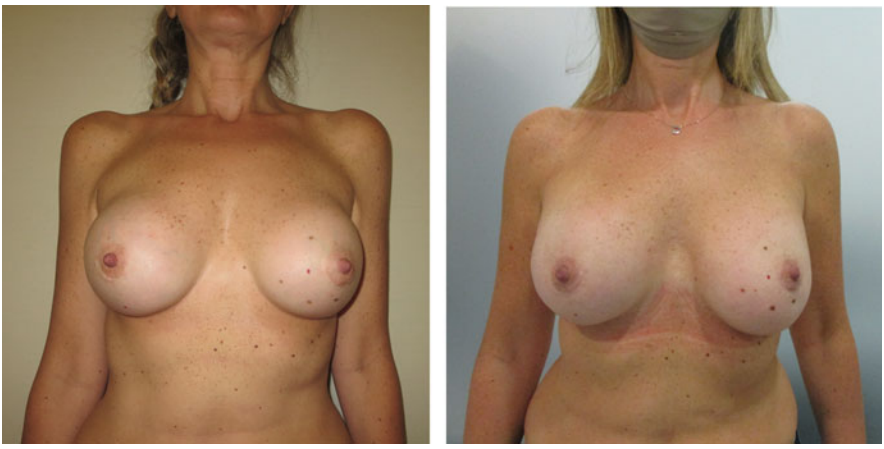


Fig. 18.3 Preoperative and postoperative outcomes after bilateral risk-reducing mastectomy and subpectoral implant reconstruction in a woman who previously underwent bilateral breast augmentation through periareolar incisions

Nowadays, submuscular reconstruction is still the first choice in skinny patients with minimal subcutaneous tissue. In these patients, the pectoralis major muscle may hide implant contour and prevent implant rippling in the upper pole.

Disadvantages of this technique include pain and discomfort in the immediate postoperative period due to muscular harvesting and dissection as well as during tissue expansion. Morbidity with serratus elevation is a further possible concern. Limited projection in the inferior pole is due to an imbalance between the submuscular pocket and the mammary skin envelope, which is often larger than the submuscular pocket. Finally, a noteworthy disadvantage associated with submuscular implant positioning is the "animation deformity" or "dancing breast". It is caused by the manipulation and contraction of the pectoralis muscle over the implant and it is often most prominent in patients who exercise frequently or lift weights.

18.1.2 Submuscular ADM-Assisted Reconstructions

An Acellular Derma Matrix (ADM) is a processed tissue graft created from either donated human cadaveric tissue or animal skin tissue. To create a graft, donated tissue goes through a series of steps to be decellularized. ADMs act as biological scaffolds, promoting angiogenesis and allowing for accelerated tissue ingrowth and cellular repopulation, thus inducing tissue regeneration. They were first utilized as dermal substitutes for the treatment of severe burns.

In breast reconstruction, the ADM can be used as an "inferior sling" sutured along the inferior border of the pectoralis major muscle for implant and soft-tissue sustenance. Therefore, the ADM gives support in the inferolateral pole, allowing the

creation of a well-defined inframammary fold, granting esthetic lateral contour and preventing implant migration.

The use of ADMs in postmastectomy reconstructions introduces the standardization of the dual-plane approach: subpectoral implant pocket in the upper pole and ADM in the inferior pole.

Submuscular ADM-assisted reconstructions may overcome some limitations of the total submuscular pocket as constricted lower pole, blunted inframammary fold, superior implant displacement, increased time for subsequent expander filling, increased pain upon expansion. In addition, reduced risk of implant malposition and reduced rate of capsular contracture are described in the literature.

Moreover, ADM extension of the muscular pocket in the inferior pole may allow the creation of larger-sized pockets, therefore facilitating direct-to-implant reconstruction.

Although still debate exists regarding the increased risk of ADM-related complications, the use of a dermal matrix seems to be associated with increased risk of seroma and infection.

Finally, the choice among different ADMs in breast reconstruction can be influenced by the market price of these biological matrices, therefore surgeons must weigh considerations of cost and patient eligibility in their decision-making process.

18.1.3 Skin-Reducing Pattern in Large and Ptotic Breasts

Postmastectomy reconstruction of large and ptotic breasts is technically challenging and it is associated with increased postoperative complications mainly related to impaired vascular supply of nipple and areola and mastectomy flaps. In these patients, a conspicuous reduction of the skin envelope is required to adjust to implant pocket and final scarring is similar to that from cosmetic surgery (inverted T scar).

In 2006, Nava et al. [7] described a single step procedure named skin-reducing mastectomy able to reconstruct large and ptotic breasts, to minimize the risk of complications and to obtain cosmetically satisfying results. The innovation of this technique consists of de-epithelialization of the area below the nipple and areola toward inframammary fold and the creation of a 1-cm-thick dermal flap, which is then sutured to the inferior border of the pectoralis major muscle (inferior dermal pedicle). The musculo-dermal implant pocket allows the use of large definitive anatomical implants in the immediate setting with good projection at the inferior pole.

Afterwards, nipple and areola can be grafted in the appropriate location, or they may be pedicled superiorly in case of shorter distance between native areolar position and new areola placement. Final scars correspond to the inverted T pattern. A contralateral mastopexy or reduction of the healthy breast is simultaneously performed to achieve good symmetry and cosmetic outcomes.

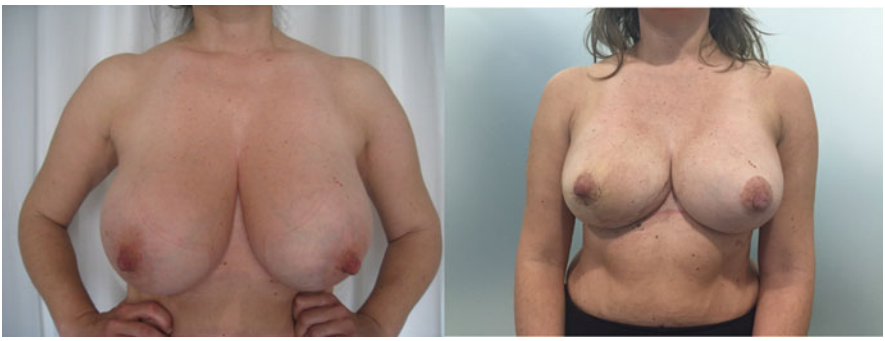


Fig. 18.4 Preoperative and postoperative outcomes after bilateral risk-reducing mastectomy and immediate reconstruction with definitive implants according to skin-reducing pattern

In very risky patients, an expander can be placed in the musculo-dermal pocket, and it can be deflated in case of mastectomy flap necrosis, allowing surgical debridement and approximation of well-vascularized edges (Fig. 18.4).

18.1.4 Prepectoral Reconstructions

According to prepectoral reconstruction, the implant is positioned subcutaneously, in the space between the pectoralis major muscle and the mastectomy skin flap; therefore, the subcutaneous layer (together with the mastectomy skin) is the sole tissue layer overlying the implant. This approach was first attempted in the 1970s. At that time, mastectomy technique was quite extensive and aggressive, skin flaps often showed impaired vascularization and necrosis and prepectoral reconstruction was abandoned because it was associated to an increased risk of implant exposure and failure.

Recent knowledge and advances toward more conservative nipple and areola-sparing mastectomies led to improved skin flap viability after oncologic resection, determining the "came-back" of the prepectoral approach.

Assessment of the subcutaneous breast layer is of utmost importance in determining whether the prepectoral approach could be a safe alternative and the first choice for breast reconstruction. Preoperative estimation of mastectomy flap thickness is mandatory using the pinch test in the upper pole. Preoperative mammograms are helpful to determine the amount of subcutaneous tissue in the upper breast pole [13] to select the right candidates for prepectoral reconstruction. In patients within minimal subcutaneous tissue, the subpectoral place is still the better solution to prevent implant rippling in the upper pole or combined procedures such as fat grafting and/or ADM placement may be needed to obtain satisfactory cosmetic outcomes.

Preoperative evaluation of comorbidities and life-style habits are similarly important to highlight those patients with an increased risk of skin necrosis after mastectomy.

However, although preoperative assessment is very important to drive indication for reconstruction, intraoperative evaluation of mastectomy skin flap and their blood supply is mandatory after the oncologic resection. Laser-assisted indocyanine green angiography is a useful tool to assess mastectomy flap perfusion intraoperatively, thereby decreasing the rate of mastectomy flap necrosis [14]. This technology allows surgeons to preserve viable and healthy tissues while balancing the need to debride poorly vascularized components of the mastectomy skin flaps.

Over the decades, the advancements in surgical technique and technology allow a safer use of the prepectoral space than in the past, outlining the advantages of this approach. Compared to submuscular implant positioning, prepectoral placement significantly reduces postoperative pain, muscle spasm, and need for pain relievers since no pectoralis muscle dissection is needed. This ultimately leads to a faster upper limb functional recovery, a faster hospital discharge and recovery after surgery, decreasing postoperative inactivity, and allowing a faster resumption of work activities. Specific cost saving data are described in the literature with regard to prepectoral reconstruction than subpectoral procedure [15]. Finally, pectoralis muscle animation is obviously avoided using the prepectoral space.

Acellular dermal matrices or synthetic meshes have been introduced in the last decades [16–18] to reduce capsular contracture and implant pressure above the mastectomy skin flap in the prepectoral space. Biological matrices may mitigate the inflammatory forces contributing to capsular contracture, serving as a barrier to the host's foreign body immune response and as a relatively inert spacer resulting in less capsular contracture. Moreover, they mechanically support the implant in the subcutaneous space, preventing pressures over the mastectomy flaps and stabilizing implant position.

However, aggressive management of postoperative complications, such as seromas and skin necrosis, is mandatory in prepectoral reconstruction to prevent implant exposure and failure.

In 2020, data on the largest multicenter study on prepectoral Braxon-assisted reconstructions have been published [19]. They include 1450 procedures and report a low rate of complications that led to implant loss in 6.5% of the cases at 5-year follow-up. Capsular contracture was associated with postoperative radiotherapy, but the overall rate was low (2.1%). In addition, even if early reconstructive algorithms excluded patients with planned radiotherapy from prepectoral implant placement because of concerns for skin dehiscence without muscle support; however, data from the iBAG study suggest that subpectoral placement avoids implant contracture by the fibrotic irradiated pectoralis major muscle, producing a more appropriate contour without the feared increase in skin dehiscence.

An alternative to matrices in the prepectoral space is represented by the use of micropolyurethane foam-coated implants, first described by De Vita et al. [20] and showing promising satisfactory results in terms of cosmetic outcomes, low complications, and morbidity [21].

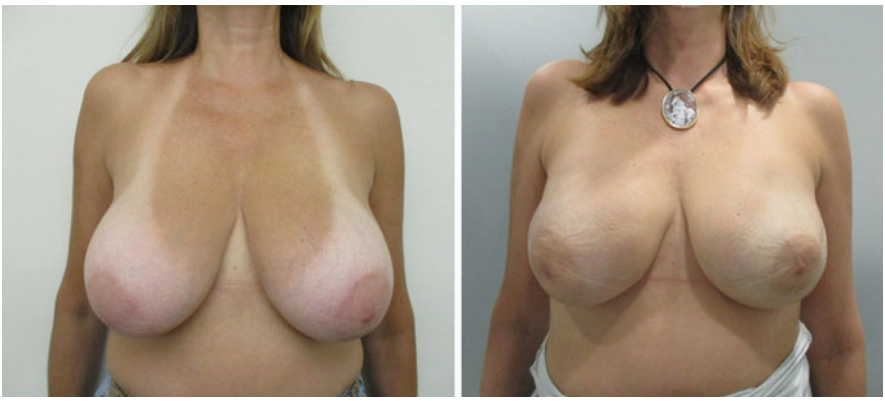


Fig. 18.5 Preoperative and postoperative results after bilateral skin-reducing mastectomy and immediate reconstruction with prepectoral micropolyurethane foam-coated implants

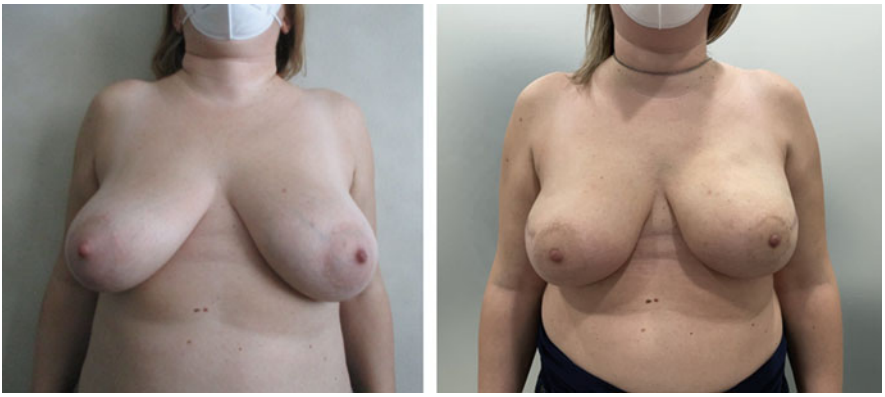


Fig. 18.6 Preoperative and postoperative results after bilateral skin-reducing mastectomy and immediate reconstruction with prepectoral micropolyurethane foam-coated implants

In large and ptotic breasts, prepectoral reconstruction may represent an alternative to skin-reducing patterns in selected cases, avoiding more sophisticated surgical approaches and related complications (Figs. 18.5 and 18.6).

18.1.5 Reconstruction After Nipple-Areola-Sparing Robotic Surgery

Robotic nipple-areola-sparing mastectomy represents an innovate approach able to enhance the visualization of the superficial mastectomy plane and providing a more precise dissection of subcutaneous tissue from glandular tissue, therefore preventing vascular compromise of mastectomy flaps. The risk of nipple and areola ischemia or

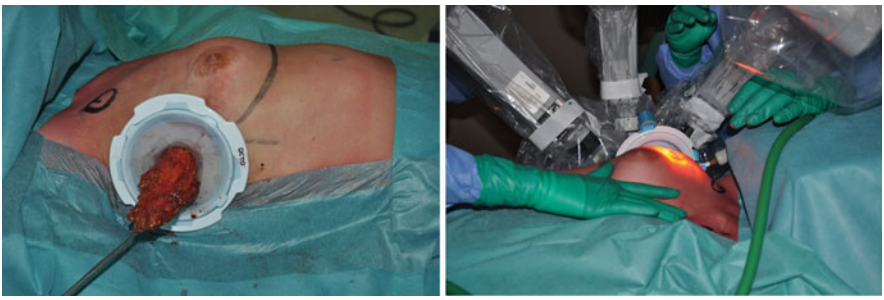


Fig. 18.7 Intraoperative views of robotic approach for risk-reducing mastectomy and reconstruction

necrosis, overall complication rate, and blood loss are therefore lower in comparison to standard surgical approach in most of the studies.

In addition, the resulting short and hidden scar perfectly responds to the rising demand of high cosmetic results by healthy women asking for risk-reducing surgery.

Reconstruction is performed with definitive implants placed both in the subpectoral place or prepectoral space according to patient anatomy and thickness of the subcutaneous layer at the upper pole of the breast.

Compared with conventional nipple-sparing mastectomy and reconstruction, robotic nipple-sparing mastectomy is associated with higher overall satisfaction and wound/scar-related outcome in patient-reported esthetic results, nevertheless longer operation time and higher overall medical cost are associated to this procedure (Fig. 18.7).

18.1.6 Combined Procedures (Fat Grafting) for Conservative Implant-Based Reconstructions

Among different types of breast reconstruction, fat grafting has been used not only as a single exclusive reconstructive technique (autologous reconstruction), but also in combination to other procedures to correct breast contour deformities and to improve overall cosmetic outcomes. In fact, although the initial scepticism surrounding the oncological safety of fat grafting in cancer patients, strong evidence demonstrated no increased risk of cancer recurrence and mortality [22].

Autologous fat transplant has been associated to improved skin trophism and vascularization and reduced postoperative pain after mastectomy.

It is frequently associated to implant-based postmastectomy reconstructions allowing camouflage of small defects, asymmetries, and visible implant borders and rippling, providing scar release and correction of skin depression and even potential reduction in capsular contracture.

It is a safe and a low-cost procedure; however, it is associated to a variable and unpredictable reabsorption rate and more procedures are sometimes necessary to achieve desired results.

Several sessions of fat grafting have been offered to postmastectomy patients with implants to improve softness at the reconstructed breast. Patients who received immediate reconstruction with expanders may progressively deflate the expander and simultaneously they can be grafted to achieve more natural results.

18.2 Cancer Patients Requiring Mastectomy at the Tumor Site and Prophylactic Mastectomy on the Healthy Breast

Although breast conservation is feasible in CDH1 mutation carriers when clinically appropriate, the vast majority of affected CDH1 carriers ask for therapeutic mastectomy and prophylactic mastectomy.

With regard to mastectomies for cancer treatment, tumor location and staging certainly influence the surgical approach and skin incisions. For those tumors superficially located, the skin over the tumor is removed within the mastectomy specimen influencing the resulting scar.

A radial skin incision at the outer quadrant of the breast is frequently performed both for glandular removal and axillary sampling [23], whether sentinel node biopsy or complete axillary dissection (Fig. 18.8).

In cases of deeply located tumors, "esthetic" skin incisions are available as well and an additional axillary incision is sometimes performed to remove axillary nodes.

Regardless of the type of mastectomy, options for reconstructions include both prostheses and autologous tissues [1].

Even in this subgroup of patients, implant-based reconstructions are more popular worldwide in comparison with autologous reconstructions [24]. In these cases, definitive silicone implants or temporary prostheses are used according to oncologic resection and the amount of spared local tissues. Less frequently, autologous flaps are necessary for reconstruction after locally advanced cancers.

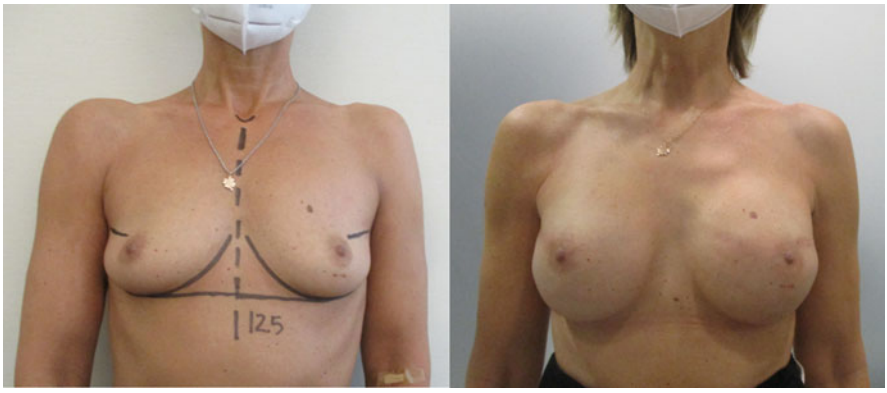


Fig. 18.8 Preoperative and postoperative results after bilateral nipple-areola-sparing mastectomy and immediate reconstruction with definitive implants

Contralateral prophylactic mastectomy can be carried out using a similar skin incision to achieve good symmetry of the reconstructed mounds; only in cases of "unfavorable" not hidden incisions at the tumor site, a different approach can be performed in the healthy breast.

18.3 Risk-Reducing Surgery After the Treatment of Primary Cancer

A heterogeneous group of women belong to this group, mainly those women who have been treated for breast cancer and at the time they were not aware about the inherited predisposition of lobular breast carcinoma. They may ask for risk-reducing surgery or they may develop a local relapse or a second tumor in the healthy breast.

Postmastectomy reconstructions after previous conservative surgery are more challenging. It occurs in those patients with hereditary gastric and breast cancer syndromes who have been treated for primary cancer with conservation and asking for risk-reducing mastectomies after genetic testing. In fact, mastectomy and reconstruction in irradiated breasts lead to higher postoperative complications due to impaired flap vascularity and healing process after radiotherapy, the presence of previous scars, and increased capsular contracture in case of implant use. It has been demonstrated that in implant-based reconstruction, radiotherapy is significantly associated with higher risk or reoperation [25]. The adverse effects of radiotherapy given both before and after implant-based reconstruction are well-documented [26].

Autologous reconstructions have been lately indicated as method of choice for irradiated breasts. However, more recently, the use of biological matrices and implants has been advocated to decrease capsular contracture rate in irradiated breasts and successfully reconstructions have been described in the literature [27, 28]. Since the two methods are not comparable in terms of safety, an accurate selection of patients for matrix-implant reconstruction in irradiated tissues is mandatory to lower postoperative complications [28].

18.3.1 Autologous Reconstruction: DIEP Flap for Bilateral Reconstructions

Although autologous reconstructions have been gaining popularity in the last decades and several studies report higher patients' satisfaction scores and quality of life in well-trained hands [29]; however, they do not represent the most popular method of reconstruction in native breasts. In fact, they often carry increased surgery-related risks, longer operating time, longer hospital stay and costs. Moreover, specific microsurgical skills are needed. Therefore, this type of reconstruction is usually reserved to selected patients, especially to those women previously treated with external breast irradiation [30].

The free Deep Inferior Epigastric Perforator (DIEP) flap is the most popular procedure among autologous breast reconstructions. The DIEP flap relies on blood

vessels (deep inferior epigastric artery and deep inferior epigastric vein) that perforate the rectus abdominus muscle to supply the overlying abdominal skin. The flap includes the abdominal skin and subcutaneous fat below the umbilicus without sacrificing the rectus abdominis muscle or rectus sheath fascia. After its transplantation to the mammary region to reconstruct the breast mound, an abdominoplasty is performed to close the donor site [31].

Patients with hereditary gastric and breast cancer syndromes who have been treated for primary cancer with conservation and irradiation and asking for risk-reducing mastectomies after genetic testing, may benefit from bilateral DIEP flap. Similarly, it occurs for those patients previously treated by conservation and requiring therapeutic bilateral mastectomies. It allows a very natural long-lasting bilateral reconstruction, assuming that subcutaneous tissue is available and well represented in the abdominal region for bilateral flaps.

Rarely, in case of bilateral risk-reducing mastectomies and previous irradiation of one breast, a monolateral DIEP flap is planned on one side and an implant-based procedure is performed in the contralateral not irradiated breast. In fact, the two methods of reconstruction act differently and breast asymmetries occur with time.

18.3.2 Latissimus Dorsi Flap and Implant

The latissimus dorsi (LD) flap has been used for the reconstruction of mastectomy defects for more than 100 years and nowadays it still represents an attractive tool for breast reconstruction [32]. Its strengths include a consistent vascular anatomy and a robust and long pedicle as well as a quick postoperative after surgery. Several refinements and indications have been described over the years, including the correction of partial breast defects after conservation, both in the immediate setting (oncoplastic replacement procedures) and in delayed setting for breast asymmetries. Autologous LD flap can be used for total breast reconstruction with or without implants, or as a salvage procedure for secondary reconstructions (implant or free-flap failure). Over the years, we have observed an evolution of oncologic surgical techniques and the development of new tools for reconstruction. LD flap has almost been abandoned for the correction of partial breast defects, asymmetries, and oncoplastic procedures. Fat grafting has replaced LD flap in the delayed setting and perforator flaps are used in the immediate setting. Nevertheless, LD reconstruction is still performed for reconstruction in irradiated breasts or after implant failure [33].

Patients with hereditary gastric and breast cancer syndromes who have been treated for primary cancer with conservation and asking for risk-reducing mastectomies after genetic testing, may benefit from implant-based reconstruction in the native not irradiated breast and LD flap with implant at the irradiated site. In this setting, an autologous flap combined with implant can reduce capsular contracture rate. Moreover, a well-vascularized muscle flap can also improve the quality and texture of the irradiated mastectomy skin.

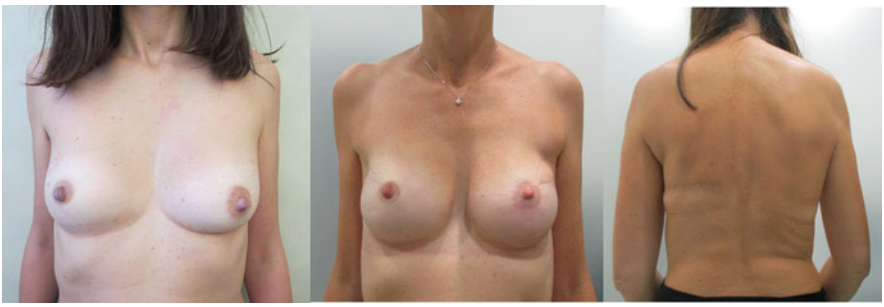


Fig. 18.9 Preoperative and postoperative results after bilateral risk-reducing mastectomy and reconstruction with implants. On the left side, a myocutaneous LD flap has been performed for reconstruction in irradiated tissues. Dorsal scar at the donor site

The implant-associated complications are generally lower than those after implant reconstruction alone and are comparable to results of two-stage expander/implant reconstructions.

In case of very tick unilateral LD flap, different sized anatomical implants are used to achieve good symmetry after bilateral mastectomies (Fig. 18.9).

18.3.3 ADM-Assisted Implant-Based Reconstructions and Prepectoral Reconstructions

Although autologous tissues represent the first choice in irradiated breast, the use of biological matrices and implants has been recently advocated to decrease capsular contracture rate in irradiated breasts [27] and successfully reconstructions have been described in the literature [19].

Matrices can be used as a sling and sutured to the inferior border of the irradiated pectoralis major muscle to create the implant pocket, otherwise the irradiated muscle is often fibrotic and inelastic, and it hinders the insertion of a definitive prosthesis.

Secondly, matrices can be used to completely wrap definitive implants positioned in the prepectoral space. In both cases, an accurate selection of patients for matrix-implant reconstruction in irradiated tissues is mandatory to lower postoperative complications. In fact, any delay in wound healing process at the mastectomy site is associated to a higher risk of wound-related complications and implant failure, since no muscle implant coverage is possible in case of matrix-assisted reconstructions.

In the Audit about prepectoral Braxon-assisted reconstructions, 198 irradiated breasts have been reconstructed using Braxon matrix in the prepectoral space. Forty-three patients received preoperative irradiation and postmastectomy Braxon-assisted prepectoral reconstruction. One hundred-fifty patients received postoperative irradiation after Braxon-assisted reconstruction. Although irradiation appears as a statistically relevant risk factor for the development of postoperative seroma, capsular

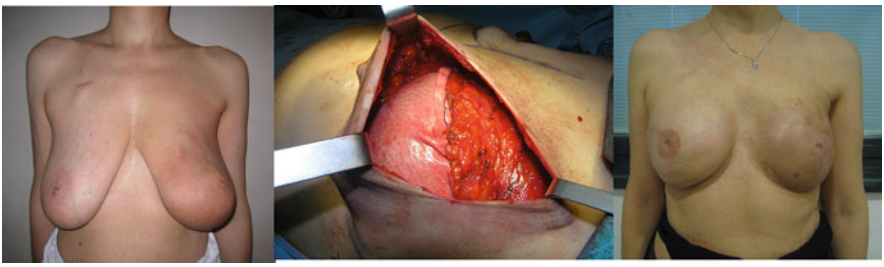


Fig. 18.10 Preoperative and postoperative results after bilateral risk-reducing mastectomy and implants in a patient previously treated with breast conservation and irradiation of the felt breast for primary cancer. On the left side, AMD has been used as an extension of the pectoralis major muscle for implant pocket

contracture, rippling, and implant loss in this cohort of patients, capsular contracture at 2-year follow-up is 5.1% and implant loss is 11.1% (Fig. 18.10).

Note
Patients' consent was obtained to publish the figures in this chapter.

References

1. Veronesi P, Ballardini B, De Lorenzi F et al (2011) Immediate breast reconstruction after mastectomy. Breast 20(Suppl 3):S104–S107. https://doi.org/10.1016/S0960-9776(11)70305-8
2. Ilonzo N, Tsang A, Tsantes S, Estabrook A, Thu Ma AM (2017) Breast reconstruction after mastectomy: a ten-year analysis of trends and immediate postoperative outcomes. Breast 32:7–12. https://doi.org/10.1016/j.breast.2016.11.023
3. Frey JD, Salibian AA, Karp NS, Choi M (2019) Implant-based breast reconstruction: hot topics, controversies, and new directions. Plast Reconstr Surg 143:404e–416e. https://doi.org/10.1097/PRS.0000000000005290
4. Kamali P, Zettervall SL, Wu W et al (2017) Differences in the reporting of racial and socioeconomic disparities among three large national databases for breast reconstruction. Plast Reconstr Surg 139:795–807. https://doi.org/10.1097/PRS.0000000000003207
5. Spear SL, Carter ME, Schwarz K (2005) Prophylactic mastectomy: indications, options, and reconstructive alternatives. Plast Reconstr Surg 115:891–909. https://doi.org/10.1097/01.prs.0000153220.66955.39
6. Majdak-Paredes EJ, Fatah F (2009) Hereditary breast cancer syndromes and clinical implications. J Plast Reconstr Aesthet Surg 62:181–189. https://doi.org/10.1016/j.bjps.2008.07.012
7. Nava MB, Cortinovis U, Ottolenghi J et al (2006) Skin-reducing mastectomy. Plast Reconstr Surg 118:603–610.; discussion 611–613. https://doi.org/10.1097/01.prs.0000233024.08392.14
8. Bertozzi N, Pesce M, Santi P, Raposio E (2017) One-stage immediate breast reconstruction: a concise review. Biomed Res Int 2017:6486859. https://doi.org/10.1155/2017/6486859
9. Santanelli di Pompeo F, Clemens MW, Atlan M et al (2022) 2022 practice recommendation updates from the World Consensus Conference on BIA-ALCL. Aesthet Surg J 42(11):1262–1278. https://doi.org/10.1093/asj/sjac133
10. Skelly A, Guo L (2021) Breast implant-associated anaplastic large cell lymphoma: a clinical update. Ann Plast Surg 86:S154–S158. https://doi.org/10.1097/SAP.0000000000002618

11. Angelos P, Bedrosian I, Euhus DM, Herrmann VM, Katz SJ, Pusic A (2015) Contralateral prophylactic mastectomy: challenging considerations for the surgeon. Ann Surg Oncol 22: 3208–3212. https://doi.org/10.1245/s10434-015-4758-y
12. Salgarello M, Barone-Adesi L, Terribile D, Masetti R (2011) Update on one-stage immediate breast reconstruction with definitive prosthesis after sparing mastectomies. Breast 20:7–14. https://doi.org/10.1016/j.breast.2010.11.005
13. Rancati A, Nava MB (2015) Latest approach to breast cancer treatment and reconstruction: conservative mastectomies. Gland Surg 4:449. https://doi.org/10.3978/j.issn.2227-684X.2015.06.10
14. Lauritzen E, Damsgaard TE (2021) Use of indocyanine green angiography decreases the risk of complications in autologous- and implant-based breast reconstruction: a systematic review and meta-analysis. J Plast Reconstr Aesthet Surg 4(8):1703–1717. https://doi.org/10.1016/j.bjps.2021.03.034
15. Cattelani L, Polotto S, Arcuri MF, Pedrazzi G, Linguadoca C, Bonati E (2018) One-step prepectoral breast reconstruction with dermal matrix-covered implant compared to submuscular implantation: functional and cost evaluation. Clin Breast Cancer 18:e703–e711. https://doi.org/10.1016/j.clbc.2017.11.015
16. Dieterich M, Dieterich H, Timme S, Reimer T, Gerber B, Stubert J (2012) Using a titanium-coated polypropylene mesh (TiLOOP(®) Bra) for implant-based breast reconstruction: case report and histological analysis. Arch Gynecol Obstet 286:273–276. https://doi.org/10.1007/s00404-012-2253-2
17. Dieterich M, Paepke S, Zwiefel K et al (2013) Implant-based breast reconstruction using a titanium-coated polypropylene mesh (TiLOOP Bra): a multicenter study of 231 cases. Plast Reconstr Surg 132:8e–19e. https://doi.org/10.1097/PRS.0b013e318290f8a0
18. Vidya R, Masià J, Cawthorn S et al (2017) Evaluation of the effectiveness of the prepectoral breast reconstruction with Braxon dermal matrix: first multicenter European report on 100 cases. Breast J 23:670–676. https://doi.org/10.1111/tbj.12810
19. Masià J, iBAG Working Group (2020) The largest multicentre data collection on prepectoral breast reconstruction: the iBAG study. J Surg Oncol 122:848–860. https://doi.org/10.1002/jso.26073
20. de Vita R, Buccheri EM, Villanucci A, Pozzi M (2019) Breast reconstruction actualized in nipple-sparing mastectomy and direct-to-implant, prepectoral polyurethane positioning: early experience and preliminary results. Clin Breast Cancer 19:e358–e363. https://doi.org/10.1016/j.clbc.2018.12.015
21. Salgarello M, Pagliara D, Barone Adesi L, Visconti G, Wild JB, Matey P (2021) Direct to implant breast reconstruction with prepectoral micropolyurethane foam-coated implant: analysis of patient satisfaction. Clin Breast Cancer 21:e454–e461. https://doi.org/10.1016/j.clbc.2021.01.015
22. Goncalves R, Mota BS, Sobreira-Lima B et al (2022) The oncological safety of autologous fat grafting: a systematic review and meta-analysis. BMC Cancer 22(1):391. https://doi.org/10.1186/s12885-022-09485-5
23. Gronet EM, Halvorson EG (2014) The oblique mastectomy incision: advantages and outcomes. Ann Plast Surg 72:S124–S125. https://doi.org/10.1097/SAP.0000000000000119
24. Albornoz CR, Bach PB, Mehrara BJ et al (2021) A paradigm shift in U.S. breast reconstruction: increasing implant rates. Plast Reconstr Surg 131(1):15–23. https://doi.org/10.1097/PRS.0b013e3182729cde
25. Unukovych D, Sandelin K, Wickman M et al (2012) Breast reconstruction in patients with personal and family history of breast cancer undergoing contralateral prophylactic mastectomy, a 10-year experience. Acta Oncol 51:934–941. https://doi.org/10.3109/0284186X.2012.666000
26. Cowen D, Gross E, Rouannet P et al (2010) Immediate post-mastectomy breast reconstruction followed by radiotherapy: risk factors for complications. Breast Cancer Res Treat 121:627–634. https://doi.org/10.1007/s10549-010-0791-5

27. Kim JYS, Mlodinow AS (2017) What's new in acellular dermal matrix and soft-tissue support for prosthetic breast reconstruction. Plast Reconstr Surg 140:30S–43S. https://doi.org/10.1097/PRS.0000000000003950
28. Gravina PR, Pettit RW, Davis MJ, Winocour SJ, Selber JC (2019) Evidence for the use of acellular dermal matrix in implant-based breast reconstruction. Semin Plast Surg 33:229–235. https://doi.org/10.1055/s-0039-1696986
29. Persichetti P, Barone M, Salzillo R et al (2022) Impact on patient's appearance perception of autologous and implant-based breast reconstruction following mastectomy using BREAST-Q. Aesthet Plast Surg 46(3):1153–1163. https://doi.org/10.1007/s00266-022-02776-z
30. Gill PS, Hunt JP, Guerra AB et al (2004) A 10-year retrospective review of 758 DIEP flaps for breast reconstruction. Plast Reconstr Surg 113:1153–1160. https://doi.org/10.1097/01.prs.0000110328.47206.50
31. Hamdi M, Weiler-Mithoff EM, Webster MH (1999) Deep inferior epigastric perforator flap in breast reconstruction: experience with the first 50 flaps. Plast Reconstr Surg 103:86–95. https://doi.org/10.1097/00006534-199901000-00015
32. De Lorenzi F, Borelli F, Sala P et al (2021) Multistage latissimus dorsi flap with implant for complex post-mastectomy reconstruction: an old but still current technique. Breast Care (Basel) 16:396–401. https://doi.org/10.1159/000511328
33. De Lorenzi F, Corso G, Botta F et al (2020) Immediate breast reconstruction with latissimus dorsi flap for patients with local recurrence of breast cancer. Eur J Surg Oncol 46:1013–1020. https://doi.org/10.1016/j.ejso.2020.01.015

Part VI

New Issues

Psychological Burden and Preferences in CDH1 Mutation Carriers: Beyond the Cancer Diagnosis

19

Marianna Masiero and Gabriella Pravettoni

Abstract

CDH1 gene mutation carriers have to deal with a significant emotional burden associated with the increased risk of developing invasive and aggressive cancer, difficulties in managing the uncertainty related to the genetic testing information, and challenging preference-sensitive decisions about risk-reducing measures might potentially affect all life trajectories. Evidence collected in this domain have stated that identifying a cancer mutation might be a disruptive event in the lives of the mutation carriers. Having a cancer mutation does not mean an increased risk for themselves, but also for their relatives who might have inherited it. Considering the complexity of the cancer mutations, it is mandatory to introduce into clinical practice tailored psychological consultation for mutation carriers and their families to help them with challenges and uncertainties connected with their condition. Here we will explore three key themes: (1) patients' experiences of uncertainty, emotional responses, and burden in CDH1 carrier's trajectory; (2) psycho-cognitive mechanisms behind the preferences' constructions in mutation carriers throughout the care pathway; and finally, (3) role of the psychological consultation for mutation carriers and their family and decision aids in clinical practice.

M. Masiero (✉) · G. Pravettoni
Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy

Applied Research Division for Cognitive and Psychological Science, IEO European Institute of Oncology, IRCCS, Milan, Italy
e-mail: Marianna.masiero@unimi.it

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_19

19.1 From the *Body* to the *Mind*: Clinical and Psychological Challenges in Gene Mutation Carriers

A growing body of evidence has highlighted [1] that mutation carriers must face an increased risk of cancer in their lifetime compared to the general population. Genetic knowledge has contributed to tailoring cancer risk and improving prevention actions by introducing *ad hoc* clinical protocols (e.g., elective surveillance, risk-reducing surgeries, and chemoprevention); however, it poses fundamental psychological and social challenges to the carriers and their families related to the management of the genetic risk information and uncertainty, treatment decisions, and long-term consequences. Considering the specific case of CDH1 mutation, carriers have to deal with an increased risk of developing hereditary diffuse gastric cancer (HDGC) and hereditary lobular breast cancer (HLBC) [1, 2]. Although the HLBC condition is rare, about 3% of families fulfilling specific criteria are CDH1 carriers. However, an increased rate of CDH1 mutation in lobular breast cancer has been observed, and often clinicians are untrained in managing it [1], particularly in dealing with the psychological consequences for mutation carriers. In these cases, referral for psychological support to handle the psychological morbidities is essential.

Moreover, mutation carriers have an increased risk of developing other cancers, including colorectal and prostate [3]. An additional element that increases the clinical complexity of this mutation and exacerbates the uncertainty is the difficulty of early identification of HDGC throughout the instrumental examination [4, 5] and, consequently, the low survival rate observed at 5 years from the diagnosis (about less than 30%). Clinical evidence indicates that this cancer develops below the lining of the stomach and displays a lack of observable symptoms. These peculiar clinical features of the tumor affect the early detection by standard screening programs and the efficacy of the antitumoral treatments [6].

These characteristics of tumors carrying the CDH1 gene mutation mean that carriers have to deal with a significant emotional burden associated with the increased risk of developing an invasive and aggressive cancer [6], difficulties in managing the uncertainty related to the genetic testing information, and challenging decisions about risk-reducing measures that might potentially affect all life trajectories and quality of life (QoL) [7, 8]. Even if risk-reducing measures such as prophylactic total gastrectomy (PTG) may contribute to the relief of anxiety related to the increased risk for HDGC, patients who underwent these procedures reported nutritional problems, fatigue that interfered with work, social and leisure activities, and body imagine concerns [9]. An analog tendency has been observed in HLBC patients who decide to undergo risk-reducing mastectomy (RRM). From a psycho-oncological perspective, mutation carriers might react to positive genetic testing in different ways; and the emotional and behavioral responses are strictly related to the inner psychological features of the individual (e.g., personality, resilience, and coping strategies), environmental and relational variables (e.g., social and family support). With this in mind, healthcare professionals working with mutation carriers must understand how individuals perceive their increased risk and differences in individual responses to the risk of disease. It requires shifting the mindset of the

healthcare professionals, where body and mind are interconnected and where "*caring for the disease*" also means *"caring for the person*" behind the cancer diagnosis. Here we will explore three key themes: (1) experiences of uncertainty, emotional responses, and burden in CDH1 carrier's trajectory; (2) psycho-cognitive mechanisms behind the preferences' constructions in mutation carriers throughout the care pathway; and finally, (3) role of the psychological consultation for mutation carriers and their family and decision aids in clinical practice.

19.1.1 The Uncertainty Hole in Cancer Genetic Testing

After learning one's genetic predisposition through genetic testing, mutation carriers experience uncertainty and fear about a future cancer diagnosis and struggle with how to protect their future health and also of their relatives. In this vein, the uncertainty is related to an increased risk of developing a secondary tumor in patients with a previous history of cancer (*survivors*) and a primary tumor for the unaffected carriers (also named as *previvors* referring to individuals who have a cancer mutation, but they have not a previous history of cancer [10]). In addition, a critical dilemma exacerbating the uncertainty and future treatment decisions is the risk of not-developing cancer [11]. Indeed, if a CDH1 carrier has an 80% probability of developing, for example HDGC, at the same time, he/she has a 20% probability of not developing gastric cancer [12]. The disease is not a "*certain event*" in mutation carriers' trajectory, but a "*probable event*" that might occur in the future. Thus, a first step to achieve a complete understanding of the mutation carriers' experience is to appreciate both the sources of the uncertainty in the genetic field and how carriers make sense of this uncertainty.

Originally, uncertainty was outlined by Brashers and colleagues (2006), "as events or situations that might be expressed as ambiguous and complex and in which the information is poor and unpredictable" [13] (p. 478). Individuals might react to the uncertainty using different emotional responses; the type of emotional response affects the following behaviors and adaptation process to the new condition. Previous research in health domains has identified three different emotional responses (negative *vs.* positive *vs.* combined) that may occur in individuals responding to the uncertainty related to the genetic risk information. First, negative emotional responses are activated when the individual perceives uncertainty as a potential danger for himself. For example, mutation carriers might feel their uncertainty as a threat and experience fear, anxiety, and worry for themselves and their relatives. Some authors have suggested that mutation carriers live underneath *"the Sword of Damocles*" [14] (p. 130), thinking that cancer might arise at any time: it is not a question of *if* cancer will occur but *when* [15] (p. 21). In the "*if condition*", the cancer is a probable event; instead of the "*when condition*", it is a certain event. Thus, carriers who think that cancer is an inevitable event in their life trajectory may experience high emotional distress such as anxiety, worry, depression, and fear of recurrence. Negative emotional responses might bring mutation carriers to seek more information about their condition (and treatment options) and manage their risks

through elective surveillance or prophylactic surgery. However, high levels of negative emotions might also bring to deny the result of genetic testing as a defensive mechanism to control the fear and worry of cancer onset or recurrence. Otherwise, uncertainty might also elicit a positive emotional response; thus, uncertainty is framed as a sort of window of opportunity prompting the patient to act, in which all are considered probable in the future. Besides, this interpretation of uncertainty might stimulate positive emotions such as optimism and consequently might promote a better adaptation and QoL. Finally, when uncertainty merges different elements, both hazard and opportunity, emotional patterns might be characterized by a synthesis of fear and excitement [13] pushing one to adopt risk behaviors. In this situation, carriers might be more prone to avoid the risk and consequently bring the inaction [13, 16].

Dean and colleagues (2016) [10, 15] highlighted that mutation carriers have to deal with three sources of uncertainty related to the information received after positive genetic testing that may impact future health decisions and associated behaviors. The first level concerns the lack of clear (or partially incomplete) scientific evidence that might cause *informational uncertainty*. While for some specific mutations, such as in breast and ovarian cancer (BRCA1/2), the clinical recommendations about treatments and information available are reliable and confirmed by a long series of clinical studies, for other mutations this is not always possible [5]. Thus, mutation carriers might not have sufficient information to decide between different treatment options such as chemoprevention *versus* active surveillance *versus* prophylactic surgical option.

For CDH1 mutation, the international scientific community has not reached a consolidated and shared consensus regarding the efficacy of electing surveillance with endoscopy for the early detection of HDGC, highlighting a lack of satisfactory sensitivity for this type of instrumental test [17]. In this situation, the patients' decision burden is profoundly amplified because the patients have conflicting information about care options; this informational uncertainty might push carriers to decide to undergo invasive and demolitive surgery, such as PTG, even if this treatment option does not reflect the patients' preferences and needs. The second level refers to *ambiguity uncertainty* that happens when the information provided by healthcare providers (e.g., by counselors, geneticists, or oncologists) is scarce and diverging from the information that the patients have collected using their personal sources of information (e.g., searching in some forum, website or discussing with relatives). This informational mismatching creates a sort of "decisional imbalance" in mutation carriers for the best care pathway to follow, increasing worry and anxiety for disease management, and future clinical outcomes. Finally, the third level refers to *stochastic uncertainty* when mutation carriers are unsure about future health outcomes connected with cancer and antitumor treatments [10]. Consistently, CDH1 mutation carriers must foresee and evaluate critical health consequences related to the treatments. For example, in the case of the PTG treatment option, patients might experience a series of long-term problems such as weight loss, reflux, dumping syndrome, diarrhea, and fatigue with a negative impact on their QoL [18]. Similarly, HLBC patients have to ponder the long-term consequences related with RRM such

as body image problems, self-esteem, and motherhood concerns. In both cases, the mental construction of the future health consequences is quite challenging. Indeed, carriers have to mentally represent the expected outcomes of a decision that might have long-term consequences. In this regard, mutation carriers at risk for HLBC and characterized by the uncertainty intolerance might decide to undergo RRM without considering the impact on their body image amply. They may experience unsatisfaction with the choice and postdecisional regret.

In conclusion, the clinical uncertainty is a principal constituent of the disease experience [19] and can never be wholly eradicated. However, carriers' uncertainty should be managed by healthcare professionals. Indeed, when uncertainty is not well managed during clinical encounters, it may provoke harmful psychosocial consequences (e.g., anxiety and depression), inadequate coping strategies, patient and doctor relationship difficulties, poor decision-making, loss of control, and lower quality of life [15, 20]. Thus, an essential step in managing the uncertainty is comprehending how carriers appraise uncertainty and which type of emotional responses are played, such as the coping strategies used to manage it [21]. In this perspective, an in-depth understanding of the psychological, social, and behavioral repercussions of the CDH1 mutation and its bearing on individual biography is essential to improve the management of the mutation carriers in clinical practice.

19.1.1.1 Emotional Responses and Psychological Consequences of Genetic Susceptibility Testing

Given the clinical complexity and seriousness of this rare syndrome and uncertainty levels, CDH1 carriers have to face a high emotional burden that might occur immediately after identifying the mutation or in the following period. From a psychological point of view, identifying a cancer mutation might symbolize a sort of *biographical disruption* [22] in the lives of the mutation carriers [23]. Michael Bury initially introduced the notion of biographical disruption, which is the diagnosis of chronic disease brings to a multilevel transformation in individuals' life. Lately, this concept has been applied to cancer mutation carriers' condition [24], highlighting that the discovery of a mutation has a significant bearing on the carriers' lives, engendering an imbalance in each system in which the individual is nested (e.g., social, family, and working context). Having a cancer mutation does not mean an increased risk for themselves but also for their relatives (e.g., sister and daughter) who might have inherited this mutation. For these reasons, some authors suggested that it is central to understand the psychological adjustment to inherited cancer risk using *systemic evolution* [25]. The disclosure of the test result to the family members can be a significant burden for the carriers, and feelings of guilt toward family members [26, 27] considering that mutations might be transmitted and might shift couple's reproductive choice.

For example, studies on BRCA1/2 mutation carriers reported that patients fear for their future health and the possibility of "transfer" of this gene mutation to their children, predisposing them to cancer and raising concerns about when to tell and test their child [14]. Also, some authors observed that mutation carriers vicariously experience the consequence of their relatives' decisions in which a mutation has

been identified [25]. For example, studies on hereditary nonpolyposis colorectal cancer patients (HNPCC) highlighted [25, 28, 29] a circular and dynamic guilt process in which patients expressed guilt for their children and their carrier siblings. In contrast, noncarrier HNPCC individuals expressed guilt to relatives with hereditary cancer. Likewise, partners of carriers might experience higher anxiety and depression, both of the risk that their partner might develop cancer and concerns about the increased risk for their children [25]. Further, mutation carriers might experience stigmatization driving them to perceive themselves as different from other people, increasing the risk of developing identity problems [27]. In this way, the discovery of a cancer mutation might bring carriers to experience feelings of loneliness and isolation [11].

Carriers' adjustment and attempts to deal with this biographical disruption might be explained by considering individual *coping strategies* and *emotional responses*. Originally, coping strategies were defined by Lazarus and Folkman as "*cognitive and behavioral efforts to manage specific external and/or internal demands that are appraised as taxing or exceeding the sources of the person*" [30] (p. 141). In the first coping model, different categories of coping strategies were defined as problem- and emotion-focused. The first one refers to the individual attitude to try to act on the problem to deal with the stressful event, while the second one refers to managing emotions elicited by the stressful event. Bennett and colleagues highlighted (2008) [31] that there are no elective coping strategies in genetic risk information, but both might help deal with this complicated situation. Implementing specific coping strategies partially depends on how the human mind elaborates health threats or dangerous cues. Miller's model [32] highlighted that individual reaction to health threats is explained by a complex relationship between an individual's mental representations, beliefs, affects, goals and values, self-regulatory strategies, interactions, and the health-relevant information [32]. In this vein, the individual response associated with discovering a genetic mutation is congruent with the mental models of the carriers and sensemaking processes used to attribute a meaning to experience [33]. Generally, positive genetic testing results might elicit different coping strategies used by carriers to cope with the increased cancer risk. Some carriers might react by changing or adopting healthy lifestyles (e.g., healthy diet, regular physical exercise, and stress-reducing measures) or adopting risk-reducing measures (e.g., following screening recommendations, performing active surveillance, or elective surgery). Otherwise, other people might develop catastrophic or fatalistic thoughts reducing the adaptation to the new situation and increasing the fear of cancer onset and anxiety.

Emotional responses to positive genetic testing might be deeply different [26] and are central in determining the individual adaptation, bolstering a better QoL, and driving care decisions. Overall, they might move from negative emotions such as fear, anger, and worry to more positive emotions such as a sense of relief or reduced anxiety and worry. Indeed, some mutation carriers might be frustrated and angry since genetic heritage cannot be modified or changed [27]; they might also be worried about the future impact on their lives. Negative emotions elicited by the situations might bring the mutation carriers to focus exclusively on the negative

aspects (e.g., *I will die of cancer*), producing distortions in risk perception (e.g., overrating their personal risk) and, consequently, suboptimal decisions [34]. Bennet and colleagues [31] highlighted that a high perception of vulnerability and not having control over their lives influence individual emotional well-being, increasing the attitude to formulate intrusive thoughts and negative future expectations and increasing distress (e.g., anxiety and depression). The type of negative thoughts and their frequencies may define emotional responses to health threats. For example, high levels of intrusive thoughts and negative expectations result in higher emotional distress [31]. On the other hand, some studies have reported that the identification of the cancer mutation might be helpful (generating positive emotions) because it has permitted a better understanding of the clinical conditions, removing doubts (e.g., *Have I or not a cancer mutation?*) and worries (e.g., *Will I develop cancer?*), to take a conscious care decision, and finally to better organize their future [11]. Coherently, Hamilton and colleagues stressed that carriers might preserve higher levels of anxiety, but they reduce cancer-related distress [4].

A key point in understanding the emotional processes is the interconnection between the attentional style and emotional responses. A growing body of evidence has highlighted that emotional responses to potential health-threatening cues might be modulated by attentional style and underlying cognitive mechanisms [35]. In particular, individuals characterized by a high monitoring style (also defined as *blunter* referring to the attitude to scan for and attend to threatening cues) are more prone to feel a higher level of emotional distress compared to individuals characterized by low monitoring (referring to the attitude to distract themselves from threat-relevant information). Thus, differences in monitoring style between subjects can explain how the individuals encode and decode a specific situation. Considerable evidence on cancer mutation carriers has consistently stated that high monitors (*information seekers*) are more inclined to develop intrusive thoughts and encode ambiguous information as highly threatening. In this way, they intensely increase perceptions of personal risk for cancer, suggesting that high monitors compared to low monitors (defined as *information avoiders*) tend to overestimate the risk of experiencing a higher emotional distress [36, 37].

Another issue that should be considered is the difference between *previvors* (defined as healthy subjects with a cancer mutation) and *survivors* (defined as patients with a previous history of cancer diagnosis) in emotional response to genetic testing [15]. More in detail, in survivors compared to the previvors, the detection of a cancer mutation might disrupt or block the adaptation process, suffering an exacerbation or reactivation of adverse feelings (e.g., anxiety, depression, worry, fear of recurrence) related to the cancer diagnosis [38] and increasing risk of recurrence. Coherently with this assumption, survivors who underwent genetic testing tend to forestall their emotional responses less precisely, thinking that; this harms QoL and reduces adherence to the treatments, clinical follow-ups, and screening recommendations [39].

These findings highlighted the critical role of emotions and coping strategies in the adaptation process following a *biographical disruption* related to positive genetic testing. In this environment, characterized by a higher uncertainty related

to their condition, mutation carriers have to make critical treatment decisions in order to manage their increased cancer risk and future health status. Thus, it is essential to understand which inner and external mechanisms are involved in carriers' decision-making processes.

19.2 Inner and External Mechanisms Behind the Construction of the Mutation Carriers' Preferences

CDH1 mutation carriers have to face challenging *preference-sensitive decisions* about risk-reducing measures (prophylactic surgery *vs.* elective surveillance) that might affect all life trajectories and QoL. The treatment decision is deeply complex and requires evaluating short- (e.g., fear of the side-effects and consequences on QoL related to the PTG and/or RRM) and long-term consequences (e.g., worry about loss of earnings, need for a family time, childbearing). From a medical perspective, genetic testing contributes to tailoring cancer risk, increasing prevention strategies, and reducing mortality; nevertheless, it increases uncertainty about treatment decisions and requires the carriers to identify their best treatment options. According to the *normative decision theory*, people should weigh the pros and cons of different treatment options by estimating each outcome's probability and utility and selecting the best option. However, individuals are not always able to do this. For example, when a carrier is informed that a mutation in CDH1 may increase the probability (e.g., respectively around 50–80% for DGC and around 40–50% for LBC compared to the healthy population without mutation) of developing DGC and LBC, such probability could be perceived as close to certainty on a psychological level. Thus, carriers might choose a more invasive treatment option in order to reduce the risk (for example, PTG or RRM) without correctly evaluating the impact of this choice on their life, increasing the risk of developing regret or inability to manage the physical and psychological consequences of such demolitive surgeries. Several studies have observed that genetic risk information is frequently transformed in "*patients' mind* [40]" (p. 734), creating a significant gap between objective and perceived risk. For example, studies on BRCA1/2 carriers have highlighted that women tend to overestimate their risks and remain up to 24% higher than their objective cancer risk [40]. This imbalance in the risk evaluation happens because individuals use a double way to interpret the risk: *risk as feelings* (respectively instinctive reactions to danger) and *risk as analysis* (respectively, logic and scientific deliberation to evaluate the risk and make a decision) [41, 42]. When people use the first way (risk as feeling) to evaluate the risk, they base their decisions on intuition, experience, or salient memories. For example, a woman with positive genetic testing for CDH1 with a friend recently diagnosed with breast cancer might evaluate their risk as higher and decide to undergo aggressive treatments such as RRM instead of active surveillance. Otherwise, the second way (risk as analysis) provides a systematic and evidence-based risk evaluation. Generally, the individuals tend to process the risk preferentially using an intuitive way, producing distortion in the risk perception and consequently sub-optimal decisions [42, 43].

Coherently with this assumption, in the *descriptive theory* of the decision, Kahneman and Tversky [44] observed that human decision-making under uncertainty is independent of a rational and objective evaluation of information. However, it is linked to the representation of the choice dilemma, which is based on individual mental construction of the reality and their *status quo* (referring to a set of elements such as knowledge, values, beliefs, and emotions). Furthermore, the mind processes the information using two distinct modules, called *system 1* (automatic, associative, and fast) and *system 2* (analytical, reason-based, verbal, and relatively slow and requiring cognitive effort). People tend to use system 1 to reduce cognitive efforts, and when problems are routine and under time constraints. In contrast, individuals use system 2 when the features of the decisional task are complex, the uncertainty is high, and there is time to reason. This double way of processing information is related to the human mind's limited cognitive and computational abilities. Indeed, according to the *Bounded Rationality Theory* by Herbert Simon, individuals are characterized by bounded rationality due to limited cognitive and computational abilities (for example limitations related to the attention, perception and memory processes) and environmental constrains. This bounded rationality brings them to use heuristic processes to face with the high number of daily decisions [45]. Heuristics are defined as mental shortcuts used by individuals to make decisions; heuristics operate throughout the experiential system (System 1) instead of the deliberative system (System 2). Although heuristics might be defined as advantageous or adaptive in specific decision-making contexts, because they allow the individuals to respond to complex information that they would be unable to process rationally, dependence on heuristics might lead to disadvantageous decisions reducing the degree to which individuals evaluate objective risks, and integrate them into their decisions [46–48].

Growing evidence has highlighted that carriers' treatment decisions might be deeply affected by these intuitive processes and led by emotions rather than by a rational evaluation of the risks and the benefits associated with the treatment options. More in detail, when the uncertainty levels are high such as in genetic testing decisions, people base their decisions on heuristics to reduce cognitive strains. In particular, health literature on decision-making has highlighted the critical role of the following heuristics: *availability*, *representative*, *affect heuristics*, and *anchoring* [41, 43, 44, 49].

In this regard, Garland and colleagues (2011) [50] highlighted that having a family member (e.g., sister, uncle, father, or mother) who died from gastric cancer or received a cancer diagnosis might prompt PTG instead of electing surveillance with endoscopy in CDH1 mutation carriers. Indeed, this event increases the salience and vividness of the association between "gastric cancer and death" and consequently causes a high degree of risk aversion, and PTG is the only treatment option considered. Similarly, the negative memories related to the negative consequences of the PTG (e.g., extreme weight loss, altered eating habits, chronic pain, and fatigue) and their impact on QoL and psychosocial sequels might act as a prompt to refuse PTG. Further, mutation carriers with no experience of gastric cancer or gastric cancer-related deaths might decide to postpone (or in some situation also to reject)

PTG [17]. This cognitive mechanism is well-known as *availability heuristics,* and it refers to the attitude of making judgments about the probability of a specific event based on how easily a case comes to mind [44].

A second mental shortcut that might be detected is called *representative heuristics*. It refers to the attitude of evaluating the probability of a specific event based on similarity with a stereotype or previous knowledge. As suggested by Peters et al. [43] (2006), the representative heuristic may better explain this process when individuals have to evaluate if women are more likely to die of breast cancer or cardiovascular disease. Generally, people are more prone to answer that the probability of dying of cardiovascular diseases, such as heart attack, is less than men. This happens because heart disease is a condition more stereotyped in men. Coherently, other studies on breast cancer patients stressed that individuals tend to feel a higher risk of developing breast cancer according to their perception of how similar they are to the "standard" woman (stereotype) who gets breast cancer [48, 51]. Thus, mutation carriers sometimes might a-priori identify who in their family will develop a genetic mutation based on similarity to an affected parent [52].

Another type of heuristic that might affect patients' treatment decisions is *anchoring*, referring to the attitude of the individuals to make a decision starting from an initial value (acting as an anchor) that consecutively is adjusted in order to provide the final decision; however, the performed adjustment is insufficient [44]. For example, a CDH1 mutation carrier with a personal experience of cancer (for example, mother died of LBC or DGC) may anchor their risk estimation at around 100%. As Peters suggested [43], the following adjustments might be made starting from 100%, but they might be lacking in obtaining a clear picture of their risk.

Finally, pioneering studies by Paul Slovic and colleagues [41, 49, 53] have introduced the *affect heuristic*, defined as a reasoning shortcut in which *affect* (sensation of "goodness" or "badness" induced by a specific cue) drives the evaluation and the selection of the information that is relevant to make a decision. Affect drives human decisions, when the decision is complex, the level of uncertainty is high, and the under-time pressure. In these conditions, human decisions are mainly driven by affective impressions instead of a rational evaluation of the situation [43]. This frame well describes the decisional environment that mutation carriers have to face. For example, patients with LBC might decide for a RRM instead of active surveillance because the fear and anxiety related to the risk of having a breast tumor go beyond an objective evaluation of the risk; without considering the consequences of this demolitive surgery on their "body and mind", and long-term QoL. On the other hand, considering the specificity of the decision to perform a PTG [54], it might be driven by the subjects' emotional activation; people who are constantly worried about getting cancer would be much more burdensome than not having a stomach.

In conclusion, behind the peculiarity of the CDH1 mutation, evidence suggests that the decision to perform prophylactic surgery or elective surveillance depends on a set of interconnected emotional, cognitive [55], and contextual factors. Considering this complexity, the international guidelines, medical and patient associations

have advocated the fundamental importance of introducing into clinical practice tailored psychological consultations [7] in order to support mutation carriers (and also their family), both in decision-making about care options and in the management of the emotional and social burden related with the discovering of CDH1 mutation using a systemic approach.

19.2.1 A Landmark in the Management of CDH1 Mutation Carriers: Introducing Psychological Consultation into Clinical Practice

The international guidelines have suggested introducing psychological support for CDH1 carriers to support risk-reducing decisions and improve emotional well-being [1, 7]. As stated by Hoskins and colleagues [17], the counseling between mutation carriers, their families, and healthcare professionals is a crucial step in the clinical management of the mutation. In particular, since the psychological burden and the multilevel factors affect treatment decisions, tailored psychological support is mandatory for the patients, their families, and primary caregivers. Indeed, a cancer mutation does not affect only the patient but also his/her family. As discussed previously, partners of mutation carriers might experience higher anxiety and depression, both due to the risk that their partner might develop cancer and concerns about the increased risk for their children [24]. Furthermore, carriers might perceive psychological and emotional burdens in communicating with their own family about positive genetic testing, and this event requires a complete "*revision of their life*". In this regard, psychological consultation should be introduced akin to standard genetic counseling but with different functions for the patients and their careers. *Genetic counseling* is a crucial component of the clinical management of patients with a CDH1 mutation. Carriers receive clinical information during genetic counseling, such as "(1) three-generation family pedigree, (2) histopathological confirmation of cancer diagnosis and/or precursor lesions, and (3) a discussion on lifespan risks of emerging DGC and LBC" [56] (p. 362). Overall, this clinical consultation mainly addresses cancer risk (both for DGC and LBC), short- and long-term consequences, treatment options, and expected outcomes. In addition, genetic counseling aims to inform carriers about the risk and benefits of genetic testing, when performed before the genetic testing. Nevertheless, genetic counseling alone is not always sufficient to inform mutation carriers and help them comprehend their preferences and needs regarding risk-reducing treatments. Carriers do not receive specific support to make a decision [57] and have reported several unmet needs.

For these reasons, it is mandatory to introduce a routine and tailored *psychological consultation* for carriers in clinical practice. The psychological support should aim to: (1) monitor carriers understanding the risk related to a cancer mutation and short- and long-term consequences, helping the carriers to develop awareness about hindering cognitive mechanisms that affect their decision and risk evaluation; (2) evaluate needs and preferences related to treatment options integrating them into clinical decision; (3) help them to cope with emotional and relational burden that might occur after the discovery of mutation; and finally (4) repair biographical

disruption in order to help carriers in becoming empowered. More in details, carriers and their families should be supported to develop a coherent and data-driven view of their risk related to the CDH1 mutation and integrate their needs and preferences into clinical decisions. Indeed, the information provided during genetic counseling is necessarily uncertain and complex. It might raise concerns in carriers about possible future changes in health status, physical and relational functioning, and QoL. It is essential to consider that prophylactic surgeries such as PTG and RRM might have iatrogenic consequences on body image, identity, sexuality, and relationships. Moreover, studies on BRCA1/2 mutation carriers have reported that women, despite surgery (RRM and oophorectomy), tend to feel at risk of developing a cancer [11] that might cause long-term psychological morbidities. Thus, an in-depth discussion about the advantages and disadvantages of prophylactic surgery should be conducted, exploring the values, preferences, and expectations associated with the treatment options, increasing the congruency between expectations and outcomes [40]. Additionally, genetic cancer-related information has a high emotional burden that might affect the individual intrinsic capacity to process the information received and finally take a decision. Individuals tend to adopt cognitive heuristics to make decisions in this specific context characterized by high emotional activation, time pressure, and uncertainty [58, 59]. Thus, psychological consultation should support carriers to face reasoning fallacies and to allow them to have a clear picture of their risk. For example, a trained psychologist might help the carriers to explore all treatment options in-depth, analyze risks and benefits, and manage their mental representations, beliefs, and expected outcomes during the consultation. So, mutation carriers will be guided to elicit their preferences and needs for each treatment, and the decision will be co-developed. In this vein, tailored psychological consultation may support carriers in coping with their known genetic risk and making *informed* and *shared decisions* with their healthcare professionals.

Consistently with this last point, existing research has reported the possibility of integrating into the clinical practice new devices named *patient decision aids* (PtDAs). The International Patient Decision Aid Standards [60] have defined the PtDAs as evidence-based devices developed to support patients to be involved in decision-making about their health [58]. PtDAs are used when patients have to face complex decisions in which more than one option is available and suitable, each of them has equal benefits and risks, the treatment options are preference-sensitive, and evidence is limited [61]. The PtDAs might have a different form (e.g., booklet, video, interactive online programs) and might be used by patients before clinical consultations or during consultations [58]. This type of device should help patients understand the risks and benefits of each treatment and their preferences and values regarding outcomes of options. Indeed, a key aspect is the capacity of such a device to integrate into the clinical decision the patient perspective. To integrate patients' values and preferences into the clinical decision, PtDAs provide treatment options in a detailed manner; thus, individuals can construct the experience (referring to the physical, emotional, and social variables associated with the expected outcomes) associated with each option. In this way, patients can understand which benefits and harms are most significant to them [61, 62].

The introduction into the clinical practice of the PtDAs might be fruitful from several points of view, such as: improving knowledge and risk perception, reducing decisional conflict and postdecisional regret, and finally permitting patients to construct their health preferences and make a decision coherent with their values and preferences [63]. Notwithstanding, as suggested by Stacey and collegues [61], for the implementation into clinical practice, some specific actions should be taken into account. Firstly, the PtDAs should be designed and developed according to patients' needs; secondly, physicians should be available and trained to use these evidence-based clinical tools and skilled in shared decision-making. Only in this manner, can the PtDAs be integrated into the clinical practice as a concrete support for patients and healthcare professionals.

19.3 Conclusions

A growing body of evidence, reviewed here, indicates that CDH1 mutation carriers must face an increased risk of cancer in their lifetime compared to the general population causing critical physical problems, psychological morbidities, and negatively impacting on QoL. In particular, mutation carriers have to face a significant emotional burden related to the increased risk of developing an invasive and aggressive cancer (or multiple cancer syndromes), difficulties in managing uncertainty, and challenging preference-sensitive decisions about risk-reducing measures affecting all life trajectories. Overall, the identification of a cancer mutation symbolizes, for the mutation carriers, a sort of biographical disruption [22], engendering an imbalance in each system in which the individual is nested.

Notwithstanding, to our knowledge poor studies are available on CDH1 mutation carriers, psychological morbidities related to their condition, and decision-making. Authors have highlighted as this type of population has several unmet needs (physical, psychological, and social) that should be considered [4, 7, 11]; and for which specific protocols should be designed and implemented in clinical practice.

The scientific community has highlighted the importance of introducing tailored psychological support for carriers to help them cope with challenges and uncertainties connected with their risk status into clinical practice [7]. As confirmed by the evidence [64–67], tailored psychological support may help carriers in decision-making about care options and managing the emotional and social burden related to the identification of a CDH1 mutation. As well as, it may contribute to reduce direct and indirect costs for healthcare professionals such as patient complaints. Further benefits can be gained, by introducing decision aids to help patients and health care providers achieve a shared and informed decision. Future research should be aimed at exploring this critical field.

References

1. Corso G, Montagna G, Figueiredo J et al (2020) Hereditary gastric and breast cancer syndromes related to cdh1 germline mutation: a multidisciplinary clinical review. Cancers 12(6):1598. https://doi.org/10.3390/CANCERS12061598
2. Mirandola S, Pellini F, Granuzzo E et al (2019) Multidisciplinary management of CDH1 germinal mutation and prophylactic management hereditary lobular breast cancer: a case report. Int J Surg Case Rep 58:92–95. https://doi.org/10.1016/j.ijscr.2019.03.053
3. Guilford P, Humar B, Blair V (2010) Hereditary diffuse gastric cancer: translation of CDH1 germline mutations into clinical practice. Gastric Cancer 13(1):1–10. https://doi.org/10.1007/S10120-009-0531-X
4. Hamilton JG, Robson ME (2019) Psychosocial effects of multigene panel testing in the context of cancer genomics. Hast Cent Rep 49(S1):S44–S52. https://doi.org/10.1002/HAST.1016
5. Lim YC, Di Pietro M, O'Donovan M et al (2014) Prospective cohort study assessing outcomes of patients from families fulfilling criteria for hereditary diffuse gastric cancer undergoing endoscopic surveillance. Gastrointest Endosc 80(1):78–87. https://doi.org/10.1016/J.GIE.2013.11.040
6. Hersperger CL, Boucher J, Theroux R (2020) Paving the way: a grounded theory of discovery and decision making for individuals with the CDH1 marker. Oncol Nurs Forum 47(4):446–456. https://doi.org/10.1188/20.ONF.446-456
7. Hamilton JG, Long JM, Brandt AC et al (2019) Patients' medical and psychosocial experiences after detection of a CDH1 variant with multigene panel testing. JCO Precis Oncol 3:1–14. https://doi.org/10.1200/PO.18.00300
8. Roberts G, Hardwick R, Fitzgerald RC (2017) Decision making, quality of life and prophylactic gastrectomy in carriers of pathogenic CDH1 mutations. Transl Gastroenterol Hepatol 2:21–21. https://doi.org/10.21037/TGH.2017.03.12
9. Prose A (2022) Scholar commons scholar commons the decision-making process for individuals at risk for the decision-making process for individuals at risk for hereditary diffuse gastric cancer hereditary diffuse gastric cancer. https://scholarcommons.sc.edu/etd. Accessed 22 Apr 2022
10. Dean M, Davidson LG (2016) Previvors' uncertainty management strategies for hereditary breast and ovarian Cancer. Health Commun 33(2):122–130. https://doi.org/10.1080/10410236.2016.1250187
11. Framp A (2010) Working toward an understanding of the impact of hereditary cancers. Gastroenterol Nurs 33(6):400–405. https://doi.org/10.1097/SGA.0B013E3182021B2A
12. Fitzgerald RC, Caldas C (2004) Clinical implications of E-cadherin associated hereditary diffuse gastric cancer. Gut 53(6):775–778. https://doi.org/10.1136/GUT.2003.022061
13. Brashers DE, Babrow A, Goldsmith D, Jackson S, Jacobs S, Neidig J (2001) Communication and uncertainty management. J Commun 51(3):477–497. https://doi.org/10.1111/J.1460-2466.2001.TB02892.X
14. DiMillo J, Samson A, Thériault A et al (2013) Living with the BRCA genetic mutation: an uncertain conclusion to an unending process. Psychol Health Med 18(2):125–134. https://doi.org/10.1080/13548506.2012.687827
15. Dean M (2016) "It's not if I get cancer, it's when I get cancer": BRCA-positive patients' (un)certain health experiences regarding hereditary breast and ovarian cancer risk. Soc Sci Med 163:21–27. https://doi.org/10.1016/j.socscimed.2016.06.039
16. Campbell-Salome G, Buchanan AH, Hallquist MLG, Rahm AK, Rocha H, Sturm AC (2021) Uncertainty management for individuals with Lynch Syndrome: identifying and responding to healthcare barriers. Patient Educ Couns 104(2):403–412. https://doi.org/10.1016/J.PEC.2020.07.017
17. Hoskins C, Tutty E, Purvis R, Shanahan M, Boussioutas A, Forrest L (2022) Young people's experiences of a CDH1 pathogenic variant: decision-making about gastric cancer risk management. J Genet Couns 31(1):242–251. https://doi.org/10.1002/jgc4.1478

18. McGarragle KM, Hart TL, Swallow C et al (2021) Barriers and facilitators to CDH1 carriers contemplating or undergoing prophylactic total gastrectomy. Familial Cancer 20(2):157–169. https://doi.org/10.1007/S10689-020-00197-Y/TABLES/4
19. McCormick KM (2002) A concept analysis of uncertainty in illness. J Nurs Scholarsh 34(2): 127–131. https://doi.org/10.1111/j.1547-5069.2002.00127.x
20. Kazer MW, Bailey DE, Sanda M, Colberg J, Kelly WK (2011) An internet intervention for management of uncertainty during active surveillance for prostate cancer. Oncol Nurs Forum 38(5):561–568. https://doi.org/10.1188/11.ONF.561-568
21. Bartley N, Napier CE, Butt Z et al (2021) Cancer patient experience of uncertainty while waiting for genome sequencing results. Front Psychol 12:647502. https://doi.org/10.3389/fpsyg.2021.647502
22. Bury M (1982) Chronic illness as biographical disruption. Sociol Health Illn 4(2):167–182. https://doi.org/10.1111/1467-9566.EP11339939
23. Radley A (2004) Making sense of illness. The social psychology of health and disease. Sage, London
24. Pranka M (2018) Biographical disruption and factors facilitating overcoming it. SHS Web Conf 51:3007. https://doi.org/10.1051/shsconf/20185103007
25. Gomes P, Pietrabissa G, Silva ER et al (2022) Family adjustment to hereditary cancer syndromes: a systematic review. Int J Environ Res Public Health 19(3). https://doi.org/10.3390/IJERPH19031603
26. Eijzenga W, Hahn DE, Aaronson NK, Kluijt I, Bleiker EM (2014) Specific psychosocial issues of individuals undergoing genetic counseling for cancer—a literature review. J Genet Couns 23(2):133–146. https://doi.org/10.1007/S10897-013-9649-4/TABLES/3
27. Witt MM, Jankowska KA (2018) Breaking bad news in genetic counseling—problems and communication tools. J Appl Genet 59(4):449–452. https://doi.org/10.1007/S13353-018-0469-Y
28. Murakami Y, Okamura H, Sugano K et al (2004) Psychologic distress after disclosure of genetic test results regarding hereditary nonpolyposis colorectal carcinoma. Cancer 101(2):395–403. https://doi.org/10.1002/CNCR.20363
29. van Oostrom I, Meijers-Heijboer H, Duivenvoorden HJ et al (2007) A prospective study of the impact of genetic susceptibility testing for BRCA1/2 or HNPCC on family relationships. Psychooncology 16(4):320–328. https://doi.org/10.1002/PON.1062
30. Folkman S, Lazarus R (1984) Stress, appraisal, and coping. Springer, New York
31. Bennett P, Wilkinson C, Turner J et al (2008) Psychological factors associated with emotional responses to receiving genetic risk information. https://doi.org/10.1007/s10897-007-9136-x
32. Miller SM, Shoda Y, Hurley K (1996) Applying cognitive-social theory to health-protective behavior: breast self-examination in cancer screening. Psychol Bull 119(1):70–94. https://doi.org/10.1037/0033-2909.119.1.70
33. Weick KE (1995) Sensemaking in organizations. Sage, Thousand Oaks
34. Mazzocco K, Masiero M, Carriero MC, Pravettoni G (2019) The role of emotions in cancer patients' decision-making. Ecancermedicalscience 13:914. https://doi.org/10.3332/ecancer.2019.914
35. Miller SM (1987) Monitoring and blunting: validation of a questionnaire to assess styles of information seeking under threat. J Pers Soc Psychol 52(2):345–353. https://doi.org/10.1037/0022-3514.52.2.345
36. Schwartz MD, Lerman C, Miller SM, Daly M, Masny A (1995) Coping disposition, perceived risk, and psychological distress among women at increased risk for ovarian cancer. Health Psychol 14(3):232–235. https://doi.org/10.1037/0278-6133.14.3.232
37. Ricci Bitti PE, Gremigni P (2013) Psicologia della Salute. Modelli teorici e contesti applicativi. In: Carocci Editore (ed) Psicologia Della Salute. Modelli Teorici e Contesti Applicativi, I° edn. Roma, p 438

38. Lombardi L, Bramanti SM, Babore A et al (2019) Psychological aspects, risk and protective factors related to BRCA genetic testing: a review of the literature. Support Care Cancer 27(10): 3647–3656. https://doi.org/10.1007/S00520-019-04918-7/TABLES/2
39. Bonadona V, Saltel P, Desseigne F et al (2022) Cancer patients who experienced diagnostic genetic testing for cancer susceptibility: reactions and behavior after the disclosure of a positive test result 1. http://aacrjournals.org/cebp/article-pdf/11/1/97/1740710/ce0102000097.pdf?casa_token=hUJP3nKSJ1AAAAAA:WVIIQEhmBYwTxAzwJTffFD_WfgSdpjPsaa1NHGIGRdr7Z1j6s_x_C-hqYgaQEz_fZyhYc2c. Accessed Mar 12 2022
40. Makhnoon S, Arun B, Bedrosian I (2022) Helping patients understand and cope with BRCA mutations. Curr Oncol Rep 24(6):733–740. https://doi.org/10.1007/s11912-022-01254-8
41. Slovic P, Peters E, Finucane ML, MacGregor DG (2005) Affect, risk, and decision making. Health Psychol 24(4 Suppl):S35–S40. https://doi.org/10.1037/0278-6133.24.4.S35
42. Slovic P, Peters E (2006) Risk perception and affect. Curr Dir Psychol Sci 15(6):322–325. https://doi.org/10.1111/j.1467-8721.2006.00461
43. Peters E, McCaul KD, Stefanek M, Nelson W (2006) A heuristics approach to understanding cancer risk perception: contributions from judgment and decision-making research. Ann Behav Med 31(1):45–52. https://doi.org/10.1207/s15324796abm3101_8
44. Tversky A, Kahneman D (1974) Judgment under uncertainty: heuristics and biases: biases in judgments reveal some heuristics of thinking under uncertainty. Science 185(4157):1124–1131. https://doi.org/10.1126/science.185.4157.1124
45. Simon HA (1990) Bounded rationality. In: Utility and Probability. Springer, Berlin, pp 15–18. https://doi.org/10.1007/978-1-349-20568-4_5
46. Peters E, Lipkus I, Diefenbach MA (2006) The functions of affect in health communications and in the construction of health preferences. J Commun 56(Suppl):S140–S162. https://doi.org/10.1111/j.1460-2466.2006.00287.x
47. Fielden HG, Brown SL, Saini P, Beesley H, Salmon P (2017) How do women at increased breast cancer risk perceive and decide between risks of cancer and risk-reducing treatments? A synthesis of qualitative research. Psychooncology 26(9):1254–1262. https://doi.org/10.1002/pon.4349
48. Pilarski R (2009) Risk perception among women at risk for hereditary breast and ovarian cancer. J Genet Couns 18(4):303–312. https://doi.org/10.1007/s10897-009-9227-y
49. Finucane ML, Alhakami A, Slovic P, Johnson SM (2000) The affect heuristic in judgments of risks and benefits. J Behav Decis Mak 13(1):1–17. https://doi.org/10.1002/(SICI)1099-0771(200001/03)13:13.0.CO;2-S
50. Garland SN, Pelletier G, Garland SN et al (2011) "How do you live without a stomach?": a multiple case study examination of total gastrectomy for palliation or prophylaxis. Palliat Support Care 9(3):305–313. https://doi.org/10.1017/S1478951511000253
51. Gerend MA, Aiken LS, West SG, Erchull MJ (2004) Beyond medical risk: investigating the psychological factors underlying women's perceptions of susceptibility to breast cancer, heart disease, and osteoporosis. Health Psychol 23(3):247–258. https://doi.org/10.1037/0278-6133.23.3.247
52. Etchegary H, Perrier C (2007) Information processing in the context of genetic risk: implications for genetic-risk communication. J Genet Couns 16(4):419–432. https://doi.org/10.1007/S10897-006-9082-Z
53. Slovic P (2001) Chapter: Cigarette smokers: rational actors or rational fools? In: Slovic P (ed) Smoking: risk, perception, and policy. Sage, Thousand Oaks, CA
54. Hallowell N, Lawton J, Badger S et al (2017) The psychosocial impact of undergoing prophylactic total gastrectomy (PTG) to manage the risk of hereditary diffuse gastric cancer (HDGC). J Genet Couns 26(4):752–762. https://doi.org/10.1007/S10897-016-0045-8
55. Hallowell N, Badger S, Richardson S et al (2016) An investigation of the factors effecting high-risk individuals' decision-making about prophylactic total gastrectomy and surveillance for hereditary diffuse gastric cancer (HDGC). Fam Cancer 15(4):665–676. https://doi.org/10.1007/s10689-016-9910-8

56. van der Post RS, Vogelaar IP, Carneiro F et al (2015) Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. J Med Genet 52(6): 361–374. https://doi.org/10.1136/JMEDGENET-2015-103094
57. Boghosian T, McCuaig JM, Carlsson L, Metcalfe KA (2021) Psychosocial interventions for women with a BRCA1 or BRCA2 mutation: a scoping review. Cancers (Basel) 13(7):1486. https://doi.org/10.3390/cancers13071486
58. McAlpine K, Lewis KB, Trevena LJ, Stacey D (2018) What is the effectiveness of patient decision aids for cancer-related decisions? A systematic review subanalysis. JCO Clin Cancer Inform 2:1–13. https://doi.org/10.1200/CCI.17.00148
59. O'Connor AM (1995) Validation of a decisional conflict scale. Med Decis Mak 15(1):25–30. https://doi.org/10.1177/0272989X9501500105
60. Elwyn G (2006) Developing a quality criteria framework for patient decision aids: online international Delphi consensus process. BMJ 333(7565):417. https://doi.org/10.1136/bmj.38926.629329.AE
61. Stacey D, Légaré F, Lewis K et al (2017) Decision aids for people facing health treatment or screening decisions. Cochrane Database Syst Rev 4(4):CD001431. https://doi.org/10.1002/14651858.CD001431.pub5
62. Stacey D, Volk RJ (2021) The international patient decision aid standards (IPDAS) collaboration: evidence update 2.0. Med Decis Mak 41(7):729–733. https://doi.org/10.1177/0272989X211035681
63. Krassuski L, Vennedey V, Stock S, Kautz-Freimuth S (2019) Effectiveness of decision aids for female BRCA1 and BRCA2 mutation carriers: a systematic review. BMC Med Inform Decis Mak 19(1):1–13. https://doi.org/10.1186/S12911-019-0872-2/TABLES/4
64. Oliveri S, Renzi C, Masiero M, Pravettoni G (2015) Living at risk: factors that affect the experience of direct-to-consumer genetic testing. Mayo Clin Proc 90(10):1323–1326. https://doi.org/10.1016/j.mayocp.2015.06.014
65. Masiero M, Cutica I, Russo S, Mazzocco K, Pravettoni G (2018) Psycho-cognitive predictors of burnout in healthcare professionals working in emergency departments. J Clin Nurs 27(13–14): 2691–2698. https://doi.org/10.1111/jocn.14376
66. Lucchiari C, Botturi A, Silvani A, Lamperti E et al (2015) Cognitive strategies and quality of life of patients with high-grade glioma. Support Care Cancer 23(12):3427–3435. https://doi.org/10.1007/s00520-015-2691-z
67. Bailo L, Guiddi P, Vergani L, Marton G, Pravettoni G (2019) The patient perspective: investigating patient empowerment enablers and barriers within the oncological care process. Ecancermedicalscience 13:912. https://doi.org/10.3332/ecancer.2019.912

Drug Repurposing in Gastric Cancer: Current Status and Future Perspectives

20

Marina Leite, Raquel Seruca, and Jorge M. Gonçalves

Abstract

Gastric cancer remains an important contributor to the global cancer burden ranking the 5th most common and the 4th most deadly cancer, according to the latest cancer statistics. Despite the recent advances in the treatment of gastric cancer, with combinatorial and targeted therapies, the overall survival and cure rates are still poor, in particular for patients with advanced metastatic disease. Several reasons may explain the yet unsatisfactory clinical outcome of gastric cancer disease, from biological to experimental and conceptual, which should be tackled in an integrated manner to allow the development of better therapeutic options. Drug repurposing (DR, also known as drug repositioning) is gaining considerable attention as an additional strategy to the mainstream de novo drug discovery process. DR provides suitable drugs to expand the cancer chemotherapy options because it may explore the vast number of approved agents with

M. Leite (✉) · R. Seruca
i3S – Instituto de Investigação e Inovação em Saúde, Epithelial Interactions in Cancer Group, Universidade do Porto, Porto, Portugal

Ipatimup – Institute of Pathology and Molecular Immunology of the University of Porto, Porto, Portugal

Faculty of Medicine, Department of Pathology, University of Porto, Porto, Portugal
e-mail: mleite@ipatimup.pt; rseruca@ipatimup.pt

J. M. Gonçalves
i3S – Instituto de Investigação e Inovação em Saúde, Epithelial Interactions in Cancer Group, Universidade do Porto, Porto, Portugal

Ipatimup – Institute of Pathology and Molecular Immunology of the University of Porto, Porto, Portugal

Faculty of Pharmacy, Laboratory of Pharmacology, Department of Drug Sciences, University of Porto, Porto, Portugal
e-mail: jgoncalves@ff.up.pt

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_20

known safety profiles. The opportunity to use repurposed drugs is grounded in the progress of the knowledge of cancer "physiology" and the consequent identification of more targetable pathways. It is also fostered by the possibility of the combined use of computational and bioinformatic tools, drug screening automation, sequencing technologies, and chemistry. Herein, after a brief introduction to gastric cancer facts and currently approved therapies, we review the current status of DR in gastric cancer mainly focusing on non-oncological drugs (i.e., drugs approved for diseases other than cancers) that have been under pre-clinical and clinical evaluation for cancer and compare the potential advantages and limitations of DR over the traditionally de novo development process. It will also be described the main strategies used to identify potentially "repurposable" drugs and discussed the challenges ahead for DR in gastric cancer.

20.1 Gastric Cancer Facts

Gastric cancer represents a major health problem, accounting for 5.6% of the global cancer incidence burden and 7.7% of all cancer-related deaths, with an estimated 1,089,103 newly diagnosed cases and 768,793 related deaths worldwide in 2020 [1, 2]. This high mortality rate is associated with the overall poor prognosis of the disease, with an average 5-year survival rate below 30%, mainly due to late diagnosis [3, 4]. The median overall survival is even lower: around 1 year, for patients diagnosed at advanced stages of the disease with distant metastases, which corresponds to more than 90% of the diagnosed patients [5].

Gastric cancer is a heterogeneous disease, comprising several tumor types. It includes gastric adenocarcinomas, which represent around 95% of all stomach cases, and several rare types, such as gastric lymphomas, mesenchymal tumors, and neuroendocrine tumors [6, 7]. Gastric adenocarcinomas are the focus of this review and will be hereafter abbreviated as GC.

20.2 Therapeutic Agents Currently Approved for Gastric Cancer

Despite the multiple and invaluable efforts of the last decades to reduce the incidence, improve therapy, and reduce mortality, GC is still a burden with yet unsatisfactory clinical outcome. Tumor resection (endoscopically or surgery) continues to be the main potential curative treatment for early and advanced localized gastric cancer. Chemotherapy, immunotherapy, or radiotherapy are used to decrease tumor volume and recurrence, and prolong the overall survival of patients. For advanced metastatic or recurrent GCs, chemotherapy becomes the mainstream treatment. It is used as a palliative to control the disease and improve patients' quality of life, though other treatment modalities can also be added to gain additional benefits [8, 9]. The number of approved therapeutic agents available to treat GC patients is scarce (see Table 20.1) when compared to those available for the treatment of other solid

Table 20.1 Approved compounds for gastric adenocarcinoma therapy by the European and/or USA regulatory authorities [10–12]

Chemical compound	Drug type	Drug category
Capecitabine	Small molecule	Antimetabolite (An oral prodrug of 5-fluorouracil, 5-FU)
Cisplatin	Small molecule	Alkylating agent Platinum-containing agent
Docetaxel	Small molecule	Antimitotic-Antimicrotubule (Taxane)
Doxorubicin	Small molecule	Anthracycline antibiotic
Fam-Trastuzumab Deruxtecan-nxki	Biological	Antibody–drug conjugate (ADC) (Humanized anti-HER2 antibody linked to a topoisomerase inhibitor, deruxtecan)
Epirubicin	Small molecule	Anthracycline antibiotic (An 4′-epi-isomer of doxorubicin)
Fluorouracil (5-FU)	Small molecule	Antimetabolite
Irinotecan	Small molecule	Topoisomerase I inhibitor (A prodrug derivative of camptothecin)
Larotrectinib	Small molecule	Tyrosine kinase inhibitor (Selective TRK inhibitor)
Mitomycin C	Small molecule	Antibiotic
Nivolumab	Biological	Immune checkpoint inhibitor monoclonal antibody (Anti-PD-1 receptor blocking antibody)
Pembrolizumab	Biological	Immune checkpoint inhibitor monoclonal antibody (Anti-PD-1 receptor blocking antibody)
Ramucirumab	Biological	Anti-angiogenic monoclonal antibody (Anti-VEGFR2 blocking antibody)
Tegafur-Gimeracil-Oteracil (S-1)	Small molecule combination	Antimetabolite combination (Tegafur, a 5-FU prodrug, plus two modulators of 5-FU metabolism, Gimeracil and Oteracil)
Trastuzumab	Biological	HER2-signaling inhibitor monoclonal antibody
Trifluridine-Tipiracil Hydrochloride (TAS-102; S95005)	Small molecule combination	Antimetabolite combination (Pyrimidine analog TFT and the thymidine phosphorylase inhibitor, TPI)

Legend: *5-FU* Fluorouracil, *HER2* Human Epidermal Growth Factor Receptor 2, *PD-1* Programmed Cell Death 1, *TFT* Pyrimidine analog 5-trifluoro-2′-deoxythymidine, *TPI* Tipiracil hydrochloride, *TRK* Tropomyosin receptor kinase, *VEGFR2* Vascular Endothelial Growth Factor Receptor 2

malignancies [13]. It comprises platinum compounds (cisplatin), fluoropyrimidines (5-FU, capecitabine, and S-1), anthracyclines (doxorubicin, and epirubicin), taxanes (paclitaxel, and docetaxel), topoisomerase I inhibitors (irinotecan), and biological drugs (anti-HER2, anti-VEGFR2, and anti-PD1) alone or in conjugates

(Fam-Trastuzumab Deruxtecan-nxki) [10]. These agents are the base of many established chemotherapeutic regimens approved for the treatment of advanced or recurrent gastric cancer patients [8, 10–12]. They are often used in combination as an attempt to increase overall survival in spite of the cost of additional toxicity [8, 14–16]. Ongoing clinical trials are investigating new doses, schemes, and combinatorial regimens that use cancer drugs approved for the treatment of other tumors [17] to improve the modest clinical gains generally obtained. Therefore, GC still remains a therapeutic challenging condition, and advances are urgently needed to expand the therapeutic options to reduce the rate of disease recurrence and mortality and extend survival and quality of life. To face this unmet therapeutic need, DR has to be considered as a valid approach.

20.3 Drug Repurposing as a Complementary Strategy to the Traditional De Novo Drug Development

In the early days, drug discovery relied exclusively on "trial-and-error" and serendipity to find compounds (drugs) that could be used to treat or cure diseases. Drugs were usually obtained from plants or other organisms. The development of chemistry allowed the isolation of the active compounds present in the complex extracts obtained from natural sources and, later, the production of the drugs in the laboratory, relieving the pressure on nature as the only source of medicines. With the increase of knowledge of chemical synthesis, the capacity to synthesize new chemical entities expanded and allowed the development of large synthetic combinatorial libraries. From the last quartile of the twentieth century onwards and fostered by biotechnology and the genome sequencing, the number of pharmacological receptors increased exponentially, allowing the identification of a huge diversity of drugs (small molecules, antibodies, antibodies–drug conjugates, gene and cellular therapies) able to interact with the new receptors, at a faster pace than ever [18–21]. This is portrayed in the IQVIA Institute's last report, showing that the global number of novel active substances launched in 2021 exceeded those of the last decade in various areas; oncology is one of them [22].

De novo drug discovery, i.e., the development of new (previously unknown) molecular entities with biological activity is grounded on two main approaches: the traditional phenotypic-based method, and the targeted-based approach [23–27]. In the phenotypic-based approach, several potential drugs are tested and those that showed efficacious in causing a given biological response in the appropriate disease model are selected. Usually the mechanism of action and targets are unknown, although they can be determined retrospectively. Targeted-based drug discovery, the most common strategy, simplified in Fig. 20.1, typically includes the identification (and validation) of a therapeutic target, which is a molecule (a pharmacological receptor) relevant for the disease, the design of the drugs to target the molecule, and the devising of an appropriate assay to access the effects of the drug/receptor interaction. After the testing of multiple potential drugs, the most promising candidates may be subjected to several rounds of optimizations and refinements in their structure until one molecule is found

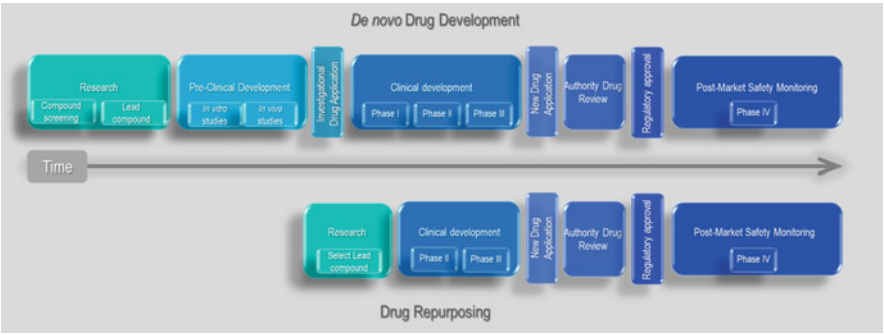


Fig. 20.1 Comparison between the drug development process required to bring a drug into the market using traditional de novo drug development versus drug repurposing approaches. In drug repurposing, the process is shorter because it bypasses or abbreviates many preapproval steps, already been performed for the original indication of the drug, at the research and development, and at the pre-clinical and safety assessment steps in phase I trial. Note that in some circumstances, phase I clinical trials can be needed in drug repurposing. Sources: [28, 29]

with the appropriate selectivity and activity profiles. Once the lead compound is optimized, it is then tested in the appropriate pre-clinical models to determine its efficacy, safety, delivery/formulation, and pharmacokinetics, using in vitro cell-based and in vivo non-human animal models.

After successful pre-clinical results, and upon the approval of an Investigational New Drug (IND) application by a drug regulatory agency, the IND needs to complete a series of clinical testing in humans (phases I, II, and III) before being authorized to be placed in the market by drug regulatory agencies. All these studies must follow the methodological procedures, namely those defined by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), to demonstrate its efficacy, safety, and benefit-risk. Even after its approval for clinical use, drug effectiveness and safety are continuously monitored (phase IV) in the context of a long time use in the real world, with a number and diversity of patients impossible to reproduce in the phase III studies. Overall, this de novo drug discovery and development process is lengthy, time-consuming, and entails a high risk of failure throughout the entire pipeline, particularly in the animal-to-human translation studies and during clinical development, when unexpected toxic effects or low clinical efficacy can occur [19, 28, 30–36]. It is estimated that, on average, it takes 10–17 years to bring an oncology medicine to the market, with a median cost of $2771.6 million. Recovery of such a huge investment implies high selling costs of the new drugs, a financial burden that might limit the access to innovative oncology treatments, at least in low- and middle-income countries [28, 37, 38]. Consequently, a new paradigm is needed to find effective and affordable oncological drugs. Exploring the potential in cancer of drugs already approved for non-oncological indications by DR is an obvious option.

DR is a term originally coined by Ashburn and Thorn to designate the process of finding new uses for already existing approved drugs [28]. Other terms with

comparable meanings have emerged since then, such as drug repositioning, drug redirecting, drug profiling, drug re-tasking, and therapeutic switching [39, 40]. The terminology may be recent but, in fact, drug repurposing has been put into practice throughout the history of medicine, as exemplified by the use of antimicrobials for cancer therapy and by the use of several cytotoxic drugs in the treatment of types of tumors different from the one for which the drug was initially approved. DR in oncology may represent an optimization of resources by shortening the costs and time to deliver a new clinical indication to drugs with known clinical safety profiles. Steps of the traditional drug development pipeline, such as the design and screening of compounds, the pre-clinical studies, and the first-in-human clinical trials (phase I), can be dispensable or abbreviated, resulting in substantial savings compared to standard de novo drug development as illustrated in Fig. 20.1.

The use of a repurposed drug in an oncology setting might still require additional phase I clinical trials to establish the effective drug dose and respective safety, which might be different from those of the original indication. If the effective drug concentration range lies within the concentration range already tested for the previous clinical indications, the development of repurposed drugs could need between 3 and 12 years, which is lower than the time needed for a normal drug development [28, 37].

The DR process is not completely absent of constraints. It can still fail in phases II and III, more likely due to lack of efficacy rather than to safety issues. In addition, technical and regulatory specificities of the drug repurposing process might delay its implementation and development, in particular, legal aspects related to intellectual property and patent ownership of repurposed drugs, and limited funding and resources to develop drug repurposing projects [37, 41].

20.4 Strategies for DR

As mentioned above, the first successful examples of repurposed drugs were discovered fortuitously. Nowadays, the search for candidate drugs for repurposing involves more systematic and rational approaches. The evolution is facilitated by the advances in technology, by increased knowledge of the biology of the disease, and, mainly, by a better understanding of the mechanisms of action of many drugs.

DR relies on two major principles, upon which several strategies are designed. First is the recognition that drugs, besides their best-known biological target (primary intended target), have several off-targets (secondary targets). Second, diseases sharing causative or disease-relevant targets could be, in principle, treated with drugs acting on the same targets and signaling pathways [42]. In fact, the advances in the knowledge of the pathophysiology of cancer are revealing that cancer shares disease-relevant pathways with other non-cancer diseases, which supports the use in cancer of drugs shown to be effective in these non-oncological diseases. In addition to this, the fact that currently approved drugs are rarely single-target agents represents a significant source of potential repurposable therapeutic agents for the treatment of diseases distinct from that of the drug's original indication in which akin targets and

associated signaling pathways might be mechanistically important [43–45]. This is particularly important in oncology given the high demand for new therapeutic drugs to overcome the resistance to the first and subsequent lines of treatment.

Drug repurposing approaches can be broadly divided into experimental-based and in silico- or computational-based methodologies (extensively detailed in [37]), both of which are increasingly used simultaneously to accelerate the repurposing process. Experimental-based approaches rely on in vitro and in vivo disease models. They are used to identify drugs that modulate (revert or stimulate) phenotypic characteristics of a given disease (phenotypic screening) or to identify the targets of a given drug for which the associated phenotypic activity was not yet mechanistically understood. Computational approaches, on the other hand, include data mining, machine learning, and network analysis of different types of drug-, disease-, and patient-related large-scale data, aiming to identify potential new drug–disease associations [46]. There is a wide diversity of computational approaches, focused on either the drug or the disease. The signature matching is the most common approach that infers similarities to identify therapeutic applications, targets, and mechanisms of action, comparing drug–drug data (e.g., shared chemical structures, target profiles, "omics" data) or drug–disease signatures (e.g., comparison of disease-associated expression profiles with and without drug treatments). Others include, for instance, the molecular docking that uses computational algorithms and structural molecular biology data to predict the binding complementarity between targets and drugs and thus identify novel interactions that can be further explored for repurposing, and the network or pathway mapping, which involves the construction of networks based on gene expression patterns, protein or disease data that is intersected with drug databases to identify potential repurposing drugs that could modulate the disease-relevant pathways [37, 47, 48].

20.5 Non-cancer Repurposed Drugs in Gastric Cancer: Pre-clinical Studies and Clinical Trials

The repurposed drugs that are currently being evaluated in the context of GC are summarized in Table 20.2. Their original therapeutic indication is presented according to the Anatomical Therapeutic Chemical (ATC) classification adopted by WHO, and as shown, is diverse, including antibiotics, antiparasitics, antipsychotics, antidepressants, and antidiabetics, among others. All are under pre-clinical or clinical research evaluation to be used in GC treatment or prevention (references of studies are indicated). Nevertheless, to date, none of the compounds have been translated into clinical practice.

Table 20.2 Non-cancer repurposed drugs investigated in pre-clinical and clinical studies for the treatment or prevention of gastric cancer

Drug name	Drug indication (ATC classification)	Pre-clinical study references	Clinical trials (study type, identifier, country, start date, status, results)
Abacavir	Anti-infectives for systemic use/Nucleoside and nucleotide reverse transcriptase inhibitors	[49]	ND
Ademetionine	Alimentary tract and metabolism/amino acids and derivatives	[50–52]	ND
Albendazole	Anthelmintics/antinematodal agents	[53, 54]	ND
Allopurinol	Musculo-skeletal system/ antigout preparations	ND	Interventional; primary purpose: Treatment – ND—[55]
Alpha-Lipoic	Alimentary tract and metabolism/various alimentary tract and metabolism products/	[56]	ND
Amiloride	Cardiovascular system/ diuretics/other potassium-sparing agents	[57, 58]	ND
Amlodipine	Cardiovascular system/ selective calcium channel blockers with mainly vascular effects	[59, 60]	ND
Anakinra	Antineoplastic and immunomodulating agents/ interleukin inhibitors	[61]	ND
Aripiprazole	Nervous system/other antipsychotics	[62]	ND
Artesunate	Antiparasitic products, insecticides, and repellents/ antimalarials	[63–65]	ND
Aspirin (Acetylsalicylic Acid)	Nervous system/other analgesics and antipyretics	[66–72, 74–81]	Observational; primary purpose: Prevention – NCT03743883; UK; 2018—[82] – NCT04081831; Hong Kong; 2019—[83] Interventional; primary purpose: Prevention – NCT04214990; Korea; 2020—Recruiting – NCT04214990;

(continued)

Table 20.2 (continued)

Drug name	Drug indication (ATC classification)	Pre-clinical study references	Clinical trials (study type, identifier, country, start date, status, results)
			Korea; 2020—Recruiting
Atenolol	Cardiovascular system/beta-blocking agents, selective	[84]	ND
Atorvastatin	Cardiovascular system/lipid-modifying agents/HMG CoA reductase inhibitors	[85, 86]	ND
Auranofin	Musculo-skeletal system/specific antirheumatic agents/gold preparations	[87, 88]	ND
Azithromycin	Anti-infectives for systemic use/macrolides, lincosamides, and streptogramins	[89]	ND
Bazedoxifene	Genito urinary system and sex hormones/Other sex hormones and modulators of the genital system/selective estrogen receptor modulators	[90]	ND
Cabergoline	Nervous system/dopamine agonists	[91]	ND
Caffeine	Nervous system/psychostimulants, agents used for ADHD and nootropics/xanthine derivatives	[92–94]	ND
Candesartan	Cardiovascular system/angiotensin II receptor blockers (ARBs)	[95–97]	ND
Cannabidiol	Nervous system/other antiepileptics	[98, 99]	ND
Captopril	Cardiovascular system/ACE inhibitors	[100]	ND
Celecoxib	Musculo-skeletal system/Anti-inflammatory and antirheumatic products, non-steroids	[64, 101–122]	Interventional; primary purpose: Treatment – ND; China—[123] – ND; China; 2004—[124] – ND; China—[125] – ND; China; 2010—[126] – ND; China; 2010—[127]

(continued)

Table 20.2 (continued)

Drug name	Drug indication (ATC classification)	Pre-clinical study references	Clinical trials (study type, identifier, country, start date, status, results)
Chloroquine	Antiparasitic products, insecticides, and repellents/ antimalarials	[128]	ND
Chlorpromazine	Nervous system/antipsychotics	[128, 129]	ND
Cimetidine	Alimentary tract and metabolism/drugs for peptic ulcer and gastro-esophageal reflux disease/H2-receptor antagonists	[130–133]	Interventional; primary purpose: Treatment – ND; Denmark; 1982—[134] – ND; UK; 1990—[135]
Colchicine	Musculo-skeletal system/ antigout preparations	[136, 137]	ND
Ciclosporin	Antineoplastic and immunomodulating agents/ calcineurin inhibitors	[138, 139]	ND
Enalapril	Cardiovascular system/ACE inhibitors, plain	[140]	ND
Deferasirox	All other therapeutic products/ iron-chelating agents	[141, 142]	ND
Deferoxamine	All other therapeutic products/ iron-chelating agents	[142]	ND
Digoxin	Cardiovascular system/digitalis glycosides	[143]	ND
Dipyridamole	Blood and blood-forming organs/platelet aggregation inhibitors excl. heparin	[144, 145]	Interventional; primary purpose: Treatment – ND; Japan—[144] – ND; Japan—[146]
Disulfiram	Nervous system/drugs used in alcohol dependence	[147–150]	ND
Doxycycline	Anti-infectives for systemic use/tetracyclines	[151, 152]	ND
Eflornithine (α-difluoro-methyl ornithine; DFMO)	Antiparasitic products, insecticides, and repellents/ other agents against leishmaniasis and trypanosomiasis	[153–162]	Interventional; primary Purpose: Prevention – NCT02794428; USA; 2016—Recruiting
Esomeprazole	Alimentary tract and metabolism/proton pump inhibitors	[163–165]	ND

(continued)

Table 20.2 (continued)

Drug name	Drug indication (ATC classification)	Pre-clinical study references	Clinical trials (study type, identifier, country, start date, status, results)
Fasudil[a]	Cardiovascular system/other peripheral vasodilators	[166]	ND
Fenofibrate	Cardiovascular system/lipid-modifying agents, plain/fibrates	[167]	ND
Fingolimod	Antineoplastic and immunomodulating agents/ selective immunosuppressants	[168]	ND
Fluoxetine	Nervous system/ antidepressants/selective serotonin reuptake inhibitors	[169–171]	ND
Glibenclamide	Alimentary tract and metabolism/blood glucose-lowering drugs, excl. insulins/ Sulfonylureas	[172]	ND
Hydroxychloroquine	Antiparasitic products, insecticides, and repellents/ antimalarials	[173]	ND
Ibuprofen	Musculo-skeletal system/anti-inflammatory and antirheumatic products, non-steroids	[77, 174, 175]	ND
Imipramine	Nervous system/ antidepressants/non-selective monoamine reuptake inhibitors	[176]	ND
Indometacin	Musculo-skeletal system/anti-inflammatory and antirheumatic products, non-steroids	[66, 67, 74, 101, 104, 177–181]	ND
Itraconazole	Anti-infectives for systemic use/antimycotics for systemic use/triazole and tetrazole derivatives	[182–184]	ND
Ivermectin	Antiparasitic products, insecticides, and repellents/ antinematodal agents/ avermectins	[182]	ND
Ketamine	Anesthetics, general/other general anesthetics	[185]	ND
Levamisole	Antiparasitic products, insecticides, and repellents/ antinematodal agents/ imidazothiazole derivatives	ND	Interventional; primary purpose: Treatment – ND; Japan; 1976—[186] – ND; Japan; 1977—[187]

(continued)

Table 20.2 (continued)

Drug name	Drug indication (ATC classification)	Pre-clinical study references	Clinical trials (study type, identifier, country, start date, status, results)
			– ND; Japan; 1979—[188] – ND; Italy; 1977—[189] – ND; Korea; 1991—[190] – ND; USA—[191]
Lercanidipine	Cardiovascular system/selective calcium channel blockers with mainly vascular effects/dihydropyridine derivatives	[60]	ND
Lidocaine	Nervous system/anesthetics, local/amides	[192–197]	ND
Linagliptin	Alimentary tract and metabolism/blood glucose-lowering drugs, excl. insulins/dipeptidyl peptidase 4 (DPP-4) inhibitors	ND	Interventional; primary purpose: treatment – NCT03281369; multi-Locations; 2017—recruiting
Lithium	Nervous system/antipsychotics	ND	Interventional; primary purpose: Treatment – NCT03153280; Ireland; 2022—Recruiting
Losartan	Cardiovascular system/angiotensin II receptor blockers (ARBs), plain	[100, 198]	ND
Lovastatin	Cardiovascular system/lipid-modifying agents, plain/HMG CoA reductase inhibitors	[145, 199–208]	Interventional; primary purpose: treatment – ND; Korea; 1996—[209]
Loxoprofen	Musculo-skeletal system/anti-inflammatory preparations, non-steroids for topical use	[210]	ND
Maraviroc	Anti-infectives for systemic use/direct-acting antivirals/other antivirals	[211]	ND
Mebendazole	Antiparasitic products, insecticides, and repellents/antinematodal agents/benzimidazole derivatives	[212–214]	ND
Mefloquine	Antiparasitic products, insecticides, and repellents/	[215]	ND

(continued)

Table 20.2 (continued)

Drug name	Drug indication (ATC classification)	Pre-clinical study references	Clinical trials (study type, identifier, country, start date, status, results)
	antimalarials/ methanolquinolines		
Melatonin	Nervous system/hypnotics and sedatives/melatonin receptor agonists	[216–233]	Interventional; primary purpose: treatment – ND; Italy—[234–236]
Metformin	Alimentary tract and metabolism/blood glucose-lowering drugs, excl. insulins/ biguanides	[237–265]	Interventional; primary purpose: treatment – NCT04033107; China; 2019—recruiting – NCT04114136; USA; 2020—recruiting
Metronidazole	Anti-infectives for systemic use/other antibacterials/ imidazole derivatives	[266]	Interventional; primary purpose: treatment – ND; Russia; 1982—[267]
Miconazole	Anti-infectives for systemic use/antimycotics for systemic use/imidazole derivatives	[268]	ND
Mifepristone	Genito urinary system and sex hormones/progesterone receptor modulators	[269, 270]	ND
Naftopidil[b]	Genito urinary system and sex hormones/drugs used in benign prostatic hypertrophy/alpha-adrenoreceptor antagonists	[271]	ND
Niclosamide	Antiparasitic products, insecticides, and repellents/ anticestodals/salicylic acid derivatives	[272]	ND
Nimesulide	Musculo-skeletal system/anti-inflammatory and antirheumatic products, non-steroids/other anti-inflammatory and antirheumatic agents, non-steroids	[273]	ND
Noscapine	Respiratory system/cough suppressants, excl. combinations with	[274]	ND

(continued)

Table 20.2 (continued)

Drug name	Drug indication (ATC classification)	Pre-clinical study references	Clinical trials (study type, identifier, country, start date, status, results)
	expectorants/opium alkaloids and derivatives		
Octreotide	Systemic hormonal preparations, excl. sex hormones and insulins/ hypothalamic hormones/ somatostatin and analogs	[69, 107, 275–280]	Interventional; primary purpose: treatment – ND; China—[123]
Olanzapine	Nervous system/ antipsychotics/Diazepines, oxazepines, thiazepines, and oxepines	ND	Interventional; primary purpose: treatment – NCT03575637; China; 2018—Unknown
Olmesartan	Cardiovascular system/agents acting on the renin-angiotensin system/angiotensin II receptor blockers (ARBs), plain	[140]	ND
Omeprazole	Alimentary tract and metabolism/proton pump inhibitors	[281]	ND
Orlistat	Alimentary tract and metabolism/peripherally acting antiobesity products	[282, 283]	ND
Ouabain	Cardiovascular system/digitalis glycosides	[284]	ND
Pantoprazole	Alimentary tract and metabolism/proton pump inhibitors	[285–291],	ND
Paroxetine	Nervous system/ antidepressants/selective serotonin reuptake inhibitors	[292]	ND
Perhexiline	Cardiovascular system/other non-selective calcium channel blockers	[293]	ND
Pioglitazone	Alimentary tract and metabolism/blood glucose-lowering drugs, excl. insulins/ thiazolidinediones	[294]	ND
Pravastatin	Cardiovascular system/lipid-modifying agents, plain/HMG CoA reductase inhibitors	ND	Interventional; primary purpose: treatment – ND; Netherlands; 2005—[295]
Propofol	Nervous system/other general anesthetics	[296–304]	ND

(continued)

Table 20.2 (continued)

Drug name	Drug indication (ATC classification)	Pre-clinical study references	Clinical trials (study type, identifier, country, start date, status, results)
Propranolol	Cardiovascular system/beta-blocking agents, non-selective	[84, 305–309]	Interventional; primary purpose: treatment – NCT04005365; China; 2019—Unknown
Rabeprazole	Alimentary tract and metabolism/proton pump inhibitors	[310]	
Ranitidine	Alimentary tract and metabolism/drugs for peptic ulcer and gastro-esophageal reflux disease (gord)/H2-receptor antagonists	ND	Interventional; primary purpose: treatment – ND; UK; 1989—[311]
Risperidone	Nervous system/antipsychotics/Other antipsychotics	[312]	ND
Ropivacaine	Nervous system/anesthetics, local/Amides	[192]	ND
Rosiglitazone	Alimentary tract and metabolism/blood glucose-lowering drugs, excl. insulins/thiazolidinediones	[313–317]	Interventional; primary purpose: treatment – NCT04114136; USA; 2020—Recruiting
Simvastatin	Cardiovascular system/lipid-modifying agents, plain/HMG CoA reductase inhibitors	[318–322]	Interventional; primary purpose: treatment – NCT01099085; Korea; 2009—[323] – NCT03086291; Korea; 2018—Recruiting – NCT01813994; Korea; 2014—Completed
Sirolimus (Rapamycin)	Antineoplastic and immunomodulating agents/selective immunosuppressants	[241, 324–331]	ND
Sitagliptin	Alimentary tract and metabolism/blood glucose-lowering drugs, excl. insulins/dipeptidyl peptidase 4 (DPP-4) inhibitors	[332]	ND
Sulfasalazine	Alimentary tract and metabolism/Intestinal anti-	[333–336]	Interventional; primary purpose:

(continued)

Table 20.2 (continued)

Drug name	Drug indication (ATC classification)	Pre-clinical study references	Clinical trials (study type, identifier, country, start date, status, results)
	inflammatory agents/ aminosalicylic acid and similar agents		treatment – ND; Japan—[337] – ND; Japan—[338]
Sertraline	Nervous system/ antidepressants/selective serotonin reuptake inhibitors	[339]	ND
Sulindac	Musculo-skeletal system/anti-inflammatory and antirheumatic products, non-steroids/acetic acid derivatives and related substances	[102, 103, 340, 341]	ND
Telmisartan	Cardiovascular system/agents acting on the renin-angiotensin system/angiotensin II receptor blockers (ARBs), plain	[342–344]	ND
Terbinafine	Dermatologicals/antifungals for systemic use	[321]	ND
Thalidomide	Antineoplastic and immunomodulating agents/ other immunosuppressants	ND	Interventional; primary purpose: treatment – NCT02401971; China; 2015—Unknown – NCT05198856; China; 2022—not yet recruiting
Thioridazine	Nervous system/ antipsychotics/Phenothiazines with piperidine structure	[345]	ND
Tigecycline	Anti-infectives for systemic use/tetracyclines	[152]	ND
Tranexamic acid	Blood and blood-forming organs/antifibrinolytics/amino acids	[199]	ND
Tranilast[c]	Respiratory system/other antihistamines for systemic use	[346–350]	ND
Ulinastatin[d]	Blood and blood-forming organs/antifibrinolytics/ proteinase inhibitors	[351]	ND
Ursodeoxycholic acid	Alimentary tract and metabolism/bile acids and derivatives	[352, 353]	ND
Valproic acid	Nervous system/antiepileptics/ fatty acid derivatives	[206, 354–357]	Interventional; primary purpose:

(continued)

Table 20.2 (continued)

Drug name	Drug indication (ATC classification)	Pre-clinical study references	Clinical trials (study type, identifier, country, start date, status, results)
			treatment – ND; Japan; 2012—[358]
Valsartan	Cardiovascular system/agents acting on the renin-angiotensin system/angiotensin II receptor blockers (ARBs), plain	[100]	ND
Verapamil	Cardiovascular system/ selective calcium channel blockers with direct cardiac effects/phenylalkylamine derivatives	[59, 359–362]	Interventional; primary purpose: treatment – ND; China; 2008—[363]
Verteporfin	Sensory organs/ antineovascularization agents/	[364–367]	ND
Vitamin C (Ascorbic Acid)	Alimentary tract and metabolism/ascorbic acid (vitamin C), incl. combinations/ascorbic acid (vitamin C), plain	[368–376]	Interventional; primary purpose: treatment – ND; China; 1994—[377] – ND; Japan; 1995—[378] – ND; China; 2017—[379] – NCT03015675; China; 2017—Unknown – NCT04033107; China; 2019—Recruiting
Vitamin D and analogs	Alimentary tract and metabolism/vitamins/vitamin D and analogs	[380–386]	Interventional; primary purpose: secondary prevention relapse – ND; Japan; 2010—[387]
Vitamin K	Blood and blood-forming organs; vitamin k and other hemostatics/vitamin K	[388, 389]	ND
Zidovudine (Azidothymidine, AZT)	Antivirals for systemic use/nucleoside and nucleotide reverse transcriptase inhibitors	[390, 391]	ND

(continued)

Table 20.2 (continued)

Drug name	Drug indication (ATC classification)	Pre-clinical study references	Clinical trials (study type, identifier, country, start date, status, results)
Zileuton	Respiratory system/other systemic drugs for obstructive airway diseases	[392, 393]	ND

Legend: Approved only in: [a]Japan and China; [b]Japan; [c]Japan and South Korea; [d]Japan, China, India, and South Korea
ND Not determined
The literature search for the pre-clinical studies was performed on the PubMed® database (https://pubmed.ncbi.nlm.nih.gov/) using the curated list of non-cancer drugs with anticancer activity from the ReDO_DB—Drug Repurposing Database (https://www.anticancerfund.org/en/redo-db;[73]) and the clinical studies were selected from the Clinical Trials Database (https://clinicaltrials.gov/) as well as on PubMed® database using the Clinical Trial filter. The therapeutic indication was defined according to the ATC classification (from the WHO Collaborating Centre for Drug Statistics Methodology)

20.6 Concluding Remarks and Future Perspectives

GC remains a deadly disease, staying far behind other malignancies in what concerns the availability of therapeutic options that significantly impact survival rates, with patients at advanced stages of the disease still having low life expectancy after diagnosis. Further research is thus urgently required, in particular, to find more therapeutic options that can be used to limit tumor growth, spread, and drug resistance, providing more effective, safer, and affordable long-term control of the disease. Until now, the number of non-cancer repurposed drugs that were investigated or are under clinical investigation for GC is limited and the results of the few clinical studies did not allow yet translation to GC of the new therapeutic candidates. The focus ahead should be also to better characterize the readily available drugs and assess them in controlled clinical trials to gather as much evidence as possible so they can be rationally included as part of therapeutic regimens in GC. Although it makes sense to explore the potential of repurposed drugs in oncology, it should be kept in mind that the conventional paradigm is based on a private investment upfront, compensated by a latter recovery of the investment. With the DR, it would hardly bring profits to directly compensate for the upfront investment and, therefore, new drug development models must also be invented to overcome the financial constraints and to expand the opportunities of clinical validation to test if DR can really bring to the market alternative medicines effective in GC.

Conflicts of Interest The authors declare no conflict of interest or financial interests to disclose.

References

1. GLOBOCAN2020 (2020) The Global Cancer Observatory (GCO). The International Agency for Research on Cancer (IARC). World Health Organization. https://gco.iarc.fr/. [cited 4 Apr 2022]
2. Sung H, Ferlay J, Siegel RL et al (2021) Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 71(3):209–249. https://doi.org/10.3322/caac.21660
3. Siegel R, Naishadham D, Jemal A (2013) Cancer statistics, 2013. CA Cancer J Clin 63(1): 11–30. https://doi.org/10.3322/caac.21166
4. Bray F, Ferlay J, Soerjomataram I et al (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68(6):394–424. https://doi.org/10.3322/caac.21492
5. Cislo M, Filip AA, Arnold Offerhaus GJ et al (2018) Distinct molecular subtypes of gastric cancer: from Lauren to molecular pathology. Oncotarget 9(27):19427–19442. https://doi.org/10.18632/oncotarget.24827
6. Ajani JA, Lee J, Sano T et al (2017) Gastric adenocarcinoma. Nat Rev Dis Prim 3:17036. https://doi.org/10.1038/nrdp.2017.36
7. Richman DM, Tirumani SH, Hornick JL et al (2017) Beyond gastric adenocarcinoma: Multimodality assessment of common and uncommon gastric neoplasms. Abdom Radiol (NY) 42(1):124–140. https://doi.org/10.1007/s00261-016-0901-x
8. Kamiya S, Rouvelas I, Lindblad M et al (2018) Current trends in gastric cancer treatment in Europe. J Cancer Metastasis Treat 4:35. https://doi.org/10.20517/2394-4722.2017.76
9. Yin S, Wang P, Xu X et al (2019) The optimal strategy of multimodality therapies for resectable gastric cancer: evidence from a network meta-analysis. J Cancer 10(14): 3094–3101. https://doi.org/10.7150/jca.30456
10. National Comprehensive Cancer Network® (2022) NCCN Guidelines for Gastric Cancer. Version 2.2020. 2020 [cited 2022 April 4]; Available from https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1434
11. Drugs Approved for Stomach (Gastric) Cancer. NIH. National Cancer Institute. https://www.cancer.gov/about-cancer/treatment/drugs/stomach
12. Digestive Cancers Europe—Drug Database for Digestive Cancers (2022). https://digestivecancers.eu/providing-support-to-patients-2/pharmaceutical-treatments/ [cited 2022 April 4]
13. National Comprehensive Cancer Network® (2022) Treatment by cancer type. [cited 2022 April 4]; Available from https://www.nccn.org/guidelines/category_1
14. Jomrich G, Schoppmann SF (2016) Targeted therapy in gastric cancer. Eur Surg 48(5): 278–284. https://doi.org/10.1007/s10353-016-0389-1
15. O Reilly DM, McLaughlin AR, Ronayne C et al (2021) Access to innovative cancer medicines in gastrointestinal oncology: 2010 through 2020. J Clin Oncol 39(3_suppl):469–469. https://doi.org/10.1200/JCO.2021.39.3_suppl.469
16. Joshi SS, Badgwell BD (2021) Current treatment and recent progress in gastric cancer. CA Cancer J Clin 71(3):264–279. https://doi.org/10.3322/caac.21657
17. United States National Institutes of Health—National Library of Medicine. Clinical Trials database: https://clinicaltrials.gov/ [database on the Internet]
18. Pina AS, Hussain A, Roque AC (2009) An historical overview of drug discovery. Methods Mol Biol 572:3–12. https://doi.org/10.1007/978-1-60761-244-5_1
19. Hughes JP, Rees S, Kalindjian SB et al (2011) Principles of early drug discovery. Br J Pharmacol 162(6):1239–1249. https://doi.org/10.1111/j.1476-5381.2010.01127.x
20. Evens R, Kaitin K (2015) The evolution of biotechnology and its impact on health care. Health Aff (Millwood) 34(2):210–219. https://doi.org/10.1377/hlthaff.2014.1023
21. Liu R, Li X, Lam KS (2017) Combinatorial chemistry in drug discovery. Curr Opin Chem Biol 38:117–126. https://doi.org/10.1016/j.cbpa.2017.03.017

22. Report Global Trends in R&D: Overview Through 2021 (2022) IQVIA Institute for Human Data Science, 10 Feb 2022, Report No
23. Gashaw I, Ellinghaus P, Sommer A et al (2012) What makes a good drug target? Drug Discov Today 17(Suppl):S24–S30. https://doi.org/10.1016/j.drudis.2011.12.008
24. Swinney DC (2013) Phenotypic vs. target-based drug discovery for first-in-class medicines. Clin Pharmacol Ther 93(4):299–301. https://doi.org/10.1038/clpt.2012.236
25. Croston GE (2017) The utility of target-based discovery. Expert Opin Drug Discov 12(5): 427–429. https://doi.org/10.1080/17460441.2017.1308351
26. Moffat JG, Vincent F, Lee JA et al (2017) Opportunities and challenges in phenotypic drug discovery: an industry perspective. Nat Rev Drug Discov 16(8):531–543. https://doi.org/10.1038/nrd.2017.111
27. Najmi A, Javed SA, Al Bratty M et al (2022) Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents. Molecules 27(2):349. https://doi.org/10.3390/molecules27020349
28. Ashburn TT, Thor KB (2004) Drug repositioning: identifying and developing new uses for existing drugs. Nat Rev Drug Discov 3(8):673–683. https://doi.org/10.1038/nrd1468
29. https://www.fda.gov/patients/learn-about-drug-and-device-approvals/drug-development-process
30. Kola I, Landis J (2004) Can the pharmaceutical industry reduce attrition rates? Nat Rev Drug Discov 3(8):711–715. https://doi.org/10.1038/nrd1470
31. Khanna I (2012) Drug discovery in pharmaceutical industry: productivity challenges and trends. Drug Discov Today 17(19-20):1088–1102. https://doi.org/10.1016/j.drudis.2012.05.007
32. Sams-Dodd F (2013) Is poor research the cause of the declining productivity of the pharmaceutical industry? An industry in need of a paradigm shift. Drug Discov Today 18(5-6): 211–217. https://doi.org/10.1016/j.drudis.2012.10.010
33. Yue WW, Froese DS, Brennan PE (2014) The role of protein structural analysis in the next generation sequencing era. Top Curr Chem 336:67–98. https://doi.org/10.1007/128_2012_326
34. Waring MJ, Arrowsmith J, Leach AR et al (2015) An analysis of the attrition of drug candidates from four major pharmaceutical companies. Nat Rev Drug Discov 14(7): 475–486. https://doi.org/10.1038/nrd4609
35. Prasad V, De Jesus K, Mailankody S (2017) The high price of anticancer drugs: origins, implications, barriers, solutions. Nat Rev Clin Oncol 14(6):381–390. https://doi.org/10.1038/nrclinonc.2017.31
36. Schneider G, Clark DE (2019) Automated de novo drug design: are we nearly there yet? Angew Chem Int Ed Engl 58(32):10792–10803. https://doi.org/10.1002/anie.201814681
37. Pushpakom S, Iorio F, Eyers PA et al (2019) Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discov 18(1):41–58. https://doi.org/10.1038/nrd.2018.168
38. Wouters OJ, McKee M, Luyten J (2020) Estimated research and development investment needed to bring a new medicine to market, 2009-2018. JAMA 323(9):844–853. https://doi.org/10.1001/jama.2020.1166
39. Langedijk J, Mantel-Teeuwisse AK, Slijkerman DS et al (2015) Drug repositioning and repurposing: terminology and definitions in literature. Drug Discov Today 20(8):1027–1034. https://doi.org/10.1016/j.drudis.2015.05.001
40. Jourdan JP, Bureau R, Rochais C et al (2020) Drug repositioning: a brief overview. J Pharm Pharmacol 72(9):1145–1151. https://doi.org/10.1111/jphp.13273
41. Oprea TI, Overington JP (2015) Computational and Practical Aspects of Drug Repositioning. Assay Drug Dev Technol 13(6):299–306. https://doi.org/10.1089/adt.2015.29011.tiodrrr
42. Gupta SC, Sung B, Prasad S et al (2013) Cancer drug discovery by repurposing: teaching new tricks to old dogs. Trends Pharmacol Sci 34(9):508–517. https://doi.org/10.1016/j.tips.2013.06.005

43. Gregori-Puigjane E, Mestres J (2008) A ligand-based approach to mining the chemogenomic space of drugs. Comb Chem High Throughput Screen 11(8):669–676. https://doi.org/10.2174/138620708785739952
44. Mestres J, Gregori-Puigjane E, Valverde S et al (2009) The topology of drug-target interaction networks: implicit dependence on drug properties and target families. Mol BioSyst 5(9): 1051–1057. https://doi.org/10.1039/b905821b
45. Lin A, Giuliano CJ, Palladino A et al (2019) Off-target toxicity is a common mechanism of action of cancer drugs undergoing clinical trials. Sci Transl Med 11(509). https://doi.org/10.1126/scitranslmed.aaw8412
46. Jarada TN, Rokne JG, Alhajj R (2020) A review of computational drug repositioning: strategies, approaches, opportunities, challenges, and directions. J Cheminform 12(1):46. https://doi.org/10.1186/s13321-020-00450-7
47. Greene CS, Voight BF (2016) Pathway and network-based strategies to translate genetic discoveries into effective therapies. Hum Mol Genet 25(R2):R94–R98. https://doi.org/10.1093/hmg/ddw160
48. Kumar S, Kumar S (2019) Chapter 6. Molecular docking: a structure-based approach for drug repurposing. In: Roy K (ed) Silico Drug Design. Academic Press, pp 161–189
49. Panneerpandian P, Devanandan HJ, Marimuthu A et al (2020) Abacavir induces the transcriptional activity of YY1 and other oncogenic transcription factors in gastric cancer cells. Antivir Res 174:104695. https://doi.org/10.1016/j.antiviral.2019.104695
50. Zhao Y, Li JS, Guo MZ et al (2010) Inhibitory effect of S-adenosylmethionine on the growth of human gastric cancer cells in vivo and in vitro. Chinese J Cancer 29(8):752–760. https://doi.org/10.5732/cjc.010.10046
51. Luo J, Li YN, Wang F et al (2010) S-adenosylmethionine inhibits the growth of cancer cells by reversing the hypomethylation status of c-myc and H-ras in human gastric cancer and colon cancer. Int J Biol Sci 6(7):784–795. https://doi.org/10.7150/ijbs.6.784
52. Da MX, Zhang YB, Yao JB et al (2014) DNA methylation regulates expression of VEGF-C, and S-adenosylmethionine is effective for VEGF-C methylation and for inhibiting cancer growth. Braz J Med Biol Res 47(12):1021–1028
53. Zhang X, Zhao J, Gao X et al (2017) Anthelmintic drug albendazole arrests human gastric cancer cells at the mitotic phase and induces apoptosis. Exp Ther Med 13(2):595–603. https://doi.org/10.3892/etm.2016.3992
54. Yang MH, Ha IJ, Um JY et al (2021) Albendazole exhibits anti-neoplastic actions against gastric cancer cells by affecting STAT3 and STAT5 activation by pleiotropic mechanism(s). Biomedicines 9(4). https://doi.org/10.3390/biomedicines9040362
55. Salim AS (1992) Oxygen-derived free-radical scavengers prolong survival in gastric cancer. Chemotherapy 38(2):135–144. https://doi.org/10.1159/000238953
56. Yang Y, Fang E, Luo J et al (2019) the antioxidant alpha-lipoic acid inhibits proliferation and invasion of human gastric cancer cells via suppression of STAT3-mediated MUC4 gene expression. Oxidative Med Cell Longev 2019:3643715. https://doi.org/10.1155/2019/3643715
57. Ding Y, Zhang H, Zhou Z et al (2012) u-PA inhibitor amiloride suppresses peritoneal metastasis in gastric cancer. World J Surg Oncol 10:270. https://doi.org/10.1186/1477-7819-10-270
58. Hosogi S, Miyazaki H, Nakajima K et al (2012) An inhibitor of Na(+)/H(+) exchanger (NHE), ethyl-isopropyl amiloride (EIPA), diminishes proliferation of MKN28 human gastric cancer cells by decreasing the cytosolic Cl(-) concentration via DIDS-sensitive pathways. Cell Physiol Biochem 30(5):1241–1253. https://doi.org/10.1159/000343315
59. Shiozaki A, Katsurahara K, Kudou M et al (2021) Amlodipine and verapamil, voltage-gated Ca(2+) channel inhibitors, suppressed the growth of gastric cancer stem cells. Ann Surg Oncol 28(9):5400–5411. https://doi.org/10.1245/s10434-021-09645-0

60. Panneerpandian P, Rao DB, Ganesan K (2021) Calcium channel blockers lercanidipine and amlodipine inhibit YY1/ERK/TGF-beta mediated transcription and sensitize the gastric cancer cells to doxorubicin. Toxicol in Vitro 74:105152. https://doi.org/10.1016/j.tiv.2021.105152
61. Gong Z, Ma J, Su H et al (2018) Interleukin-1 receptor antagonist inhibits angiogenesis in gastric cancer. Int J Clin Oncol 23(4):659–670. https://doi.org/10.1007/s10147-018-1242-2
62. Shokrzadeh M, Mohammadpour A, Modanloo M et al (2019) Cytotoxic effects of aripiprazole on Mkn45 and Nih3t3 cell lines and genotoxic effects on human peripheral blood lymphocytes. Arq Gastroenterol 56(2):155–159. https://doi.org/10.1590/S0004-2803.201900000-31
63. Zhou X, Sun WJ, Wang WM et al (2013) Artesunate inhibits the growth of gastric cancer cells through the mechanism of promoting oncosis both in vitro and in vivo. Anti-Cancer Drugs 24(9):920–927. https://doi.org/10.1097/CAD.0b013e328364a109
64. Zhang P, Luo HS, Li M et al (2015) Artesunate inhibits the growth and induces apoptosis of human gastric cancer cells by downregulating COX-2. Onco Targets Ther 8:845–854. https://doi.org/10.2147/OTT.S81041
65. Wang L, Liu L, Wang J et al (2017) Inhibitory effect of artesunate on growth and apoptosis of gastric cancer cells. Arch Med Res 48(7):623–630. https://doi.org/10.1016/j.arcmed.2018.03.004
66. Zhu GH, Wong BC, Eggo MC et al (1999) Non-steroidal anti-inflammatory drug-induced apoptosis in gastric cancer cells is blocked by protein kinase C activation through inhibition of c-myc. Br J Cancer 79(3-4):393–400. https://doi.org/10.1038/sj.bjc.6690062
67. Zhou XM, Wong BC, Fan XM et al (2001) Non-steroidal anti-inflammatory drugs induce apoptosis in gastric cancer cells through up-regulation of bax and bak. Carcinogenesis 22(9):1393–1397. https://doi.org/10.1093/carcin/22.9.1393
68. Azarschab P, Al-Azzeh E, Kornberger W et al (2001) Aspirin promotes TFF2 gene activation in human gastric cancer cell lines. FEBS Lett 488(3):206–210. https://doi.org/10.1016/s0014-5793(00)02422-4
69. Tang C, Wang C, Tang L (2003) Effects of combined octreotide and aspirin on the growth of gastric cancer. Chin Med J 116(3):373–377
70. Gu Q, Wang JD, Xia HH et al (2005) Activation of the caspase-8/Bid and Bax pathways in aspirin-induced apoptosis in gastric cancer. Carcinogenesis 26(3):541–546. https://doi.org/10.1093/carcin/bgh345
71. Becker JC, Muller-Tidow C, Stolte M et al (2006) Acetylsalicylic acid enhances antiproliferative effects of the EGFR inhibitor gefitinib in the absence of activating mutations in gastric cancer. Int J Oncol 29(3):615–623. https://doi.org/10.3892/ijo.29.3.615
72. Redlak MJ, Power JJ, Miller TA (2007) Aspirin-induced apoptosis in human gastric cancer epithelial cells: relationship with protein kinase C signaling. Dig Dis Sci 52(3):810–816. https://doi.org/10.1007/s10620-006-9577-3
73. Pantziarka P, Verbaanderd C, Sukhatme V et al (2018) ReDO_DB: the repurposing drugs in oncology database. Ecancermedicalscience 12:886. https://doi.org/10.3332/ecancer.2018.886
74. Zhang YJ, Dai Q, Wu SM et al (2008) Susceptibility for NSAIDs-induced apoptosis correlates to p53 gene status in gastric cancer cells. Cancer Investig 26(9):868–877. https://doi.org/10.1080/07357900801944872
75. Yang L, Zhu H, Liu D et al (2011) Aspirin suppresses growth of human gastric carcinoma cell by inhibiting survivin expression. J Biomed Res 25(4):246–253. https://doi.org/10.1016/S1674-8301(11)60033-X
76. Dong H, Liu G, Jiang B et al (2014) The effects of aspirin plus cisplatin on SGC7901/CDDP cells in vitro. Biomed Rep 2(3):344–348. https://doi.org/10.3892/br.2014.241
77. Akrami H, Aminzadeh S, Fallahi H (2015) Inhibitory effect of ibuprofen on tumor survival and angiogenesis in gastric cancer cell. Tumour Biol 36(5):3237–3243. https://doi.org/10.1007/s13277-014-2952-3

78. Li XF, Xu BZ, Wang SZ (2016) Aspirin inhibits the proliferation and migration of gastric cancer cells in p53-knockout mice. Oncol Lett 12(5):3183–3186. https://doi.org/10.3892/ol.2016.5067
79. Mikami J, Kurokawa Y, Takahashi T et al (2016) Antitumor effect of antiplatelet agents in gastric cancer cells: an in vivo and in vitro study. Gastric Cancer 19(3):817–826. https://doi.org/10.1007/s10120-015-0556-2
80. Zhang W, Tan Y, Ma H (2017) Combined aspirin and apatinib treatment suppresses gastric cancer cell proliferation. Oncol Lett 14(5):5409–5417. https://doi.org/10.3892/ol.2017.6858
81. Shah S, Pocard M, Mirshahi M (2019) Targeting the differentiation of gastric cancer cells (KATOIII) downregulates epithelial mesenchymal and cancer stem cell markers. Oncol Rep 42(2):670–678. https://doi.org/10.3892/or.2019.7198
82. Bayer (2020) EffectiveNess of low-dose Aspirin in GastrointEstinal cancer prevention – United Kingdom ("ENgAGE—UK"): a cohort study on the risk of gastric and oesophageal cancer among new users of low-dose Aspirin using the THIN database in the UK—observational study results synopsis (ID20326). Report no
83. Shami JJP, Zhao J, Pathadka S et al (2022) Safety and effectiveness of low-dose aspirin for the prevention of gastrointestinal cancer in adults without atherosclerotic cardiovascular disease: a population-based cohort study. BMJ Open 12(2):e050510. https://doi.org/10.1136/bmjopen-2021-050510
84. Zhang X, Zhang Y, He Z et al (2019) Chronic stress promotes gastric cancer progression and metastasis: an essential role for ADRB2. Cell Death Dis 10(11):788. https://doi.org/10.1038/s41419-019-2030-2
85. Lee S, Lee HJ, Kang H et al (2019) Trastuzumab induced chemobrain, atorvastatin rescued chemobrain with enhanced anticancer effect and without hair loss-side effect. J Clin Med 8(2):234. https://doi.org/10.3390/jcm8020234
86. Wei J, Peng K, Zhu J et al (2020) Geranylgeranylation promotes proliferation, migration and invasion of gastric cancer cells through the YAP signaling pathway. Am J Transl Res 12(9):5296–5307
87. Zou P, Chen M, Ji J et al (2015) Auranofin induces apoptosis by ROS-mediated ER stress and mitochondrial dysfunction and displayed synergistic lethality with piperlongumine in gastric cancer. Oncotarget 6(34):36505–36521. https://doi.org/10.18632/oncotarget.5364
88. Kim TW, Lee SJ, Kim JT et al (2016) Kallikrein-related peptidase 6 induces chemotherapeutic resistance by attenuating auranofin-induced cell death through activation of autophagy in gastric cancer. Oncotarget 7(51):85332–85348. https://doi.org/10.18632/oncotarget.13352
89. Zhou X, Zhang Y, Li Y et al (2012) Azithromycin synergistically enhances anti-proliferative activity of vincristine in cervical and gastric cancer cells. Cancers 4(4):1318–1332. https://doi.org/10.3390/cancers4041318
90. Thilakasiri P, Huynh J, Poh AR et al (2019) Repurposing the selective estrogen receptor modulator bazedoxifene to suppress gastrointestinal cancer growth. EMBO Mol Med 11(4). https://doi.org/10.15252/emmm.201809539
91. Leng ZG, Lin SJ, Wu ZR et al (2017) Activation of DRD5 (dopamine receptor D5) inhibits tumor growth by autophagic cell death. Autophagy 13(8):1404–1419. https://doi.org/10.1080/15548627.2017.1328347
92. Takahashi M, Yanoma S, Yamamoto Y et al (1998) Combined effect of CDDP and caffeine against human gastric cell line in vivo. Anticancer Res 18(6A):4399–4401
93. Liu H, Zhou Y, Tang L (2017) Caffeine induces sustained apoptosis of human gastric cancer cells by activating the caspase 9/caspase 3 signalling pathway. Mol Med Rep 16(3):2445–2454. https://doi.org/10.3892/mmr.2017.6894
94. Liu H, Song J, Zhou Y et al (2019) Methylxanthine derivatives promote autophagy in gastric cancer cells targeting PTEN. Anti-Cancer Drugs 30(4):347–355. https://doi.org/10.1097/CAD.0000000000000724

95. Huang W, Wu YL, Zhong J et al (2008) Angiotensin II type 1 receptor antagonist suppress angiogenesis and growth of gastric cancer xenografts. Dig Dis Sci 53(5):1206–1210. https://doi.org/10.1007/s10620-007-0009-9
96. Kinoshita J, Fushida S, Harada S et al (2009) Local angiotensin II-generation in human gastric cancer: correlation with tumor progression through the activation of ERK1/2, NF-kappaB and survivin. Int J Oncol 34(6):1573–1582. https://doi.org/10.3892/ijo_00000287
97. Okazaki M, Fushida S, Harada S et al (2014) The angiotensin II type 1 receptor blocker candesartan suppresses proliferation and fibrosis in gastric cancer. Cancer Lett 355(1):46–53. https://doi.org/10.1016/j.canlet.2014.09.019
98. Zhang X, Qin Y, Pan Z et al (2019) Cannabidiol induces cell cycle arrest and cell apoptosis in human gastric cancer SGC-7901 cells. Biomolecules 9(8). https://doi.org/10.3390/biom9080302
99. Jeong S, Jo MJ, Yun HK et al (2019) Cannabidiol promotes apoptosis via regulation of XIAP/Smac in gastric cancer. Cell Death Dis 10(11):846. https://doi.org/10.1038/s41419-019-2001-7
100. Wang L, Cai SR, Zhang CH et al (2008) Effects of angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor blockers on lymphangiogenesis of gastric cancer in a nude mouse model. Chin Med J 121(21):2167–2171
101. Hu PJ, Yu J, Zeng ZR et al (2004) Chemoprevention of gastric cancer by celecoxib in rats. Gut 53(2):195–200. https://doi.org/10.1136/gut.2003.021477
102. Fu SL, Wu YL, Zhang YP et al (2004) Anti-cancer effects of COX-2 inhibitors and their correlation with angiogenesis and invasion in gastric cancer. World J Gastroenterol 10(13):1971–1974. https://doi.org/10.3748/wjg.v10.i13.1971
103. Wu YL, Fu SL, Zhang YP et al (2005) Cyclooxygenase-2 inhibitors suppress angiogenesis and growth of gastric cancer xenografts. Biomed Pharmacother 59(Suppl 2):S289–S292. https://doi.org/10.1016/s0753-3322(05)80048-4
104. Yu J, Tang BD, Leung WK et al (2005) Different cell kinetic changes in rat stomach cancer after treatment with celecoxib or indomethacin: implications on chemoprevention. World J Gastroenterol 11(1):41–45. https://doi.org/10.3748/wjg.v11.i1.41
105. Cho SJ, Kim N, Kim JS et al (2007) The anti-cancer effect of COX-2 inhibitors on gastric cancer cells. Dig Dis Sci 52(7):1713–1721. https://doi.org/10.1007/s10620-007-9787-3
106. Pang RP, Zhou JG, Zeng ZR et al (2007) Celecoxib induces apoptosis in COX-2 deficient human gastric cancer cells through Akt/GSK3beta/NAG-1 pathway. Cancer Lett 251(2):268–277. https://doi.org/10.1016/j.canlet.2006.11.032
107. Wang CH, Zheng WB, Qiang O et al (2009) Effects of non-cytotoxic drugs on the growth of multidrug-resistance human gastric carcinoma cell line. J Dig Dis 10(2):91–98. https://doi.org/10.1111/j.1751-2980.2009.00370.x
108. Saito Y, Suzuki H, Imaeda H et al (2013) The tumor suppressor microRNA-29c is downregulated and restored by celecoxib in human gastric cancer cells. Int J Cancer 132(8):1751–1760. https://doi.org/10.1002/ijc.27862
109. Wang YJ, Niu XP, Yang L et al (2013) Effects of celecoxib on cycle kinetics of gastric cancer cells and protein expression of cytochrome C and caspase-9. Asian Pac J Cancer Prev 14(4):2343–2347. https://doi.org/10.7314/apjcp.2013.14.4.2343
110. Liu T, Liang X, Li B et al (2013) Telomerase reverse transcriptase inhibition stimulates cyclooxygenase 2 expression in cancer cells and synergizes with celecoxib to exert anti-cancer effects. Br J Cancer 108(11):2272–2280. https://doi.org/10.1038/bjc.2013.208
111. Chen M, Yu L, Gu C et al (2013) Celecoxib antagonizes the cytotoxic effect of cisplatin in human gastric cancer cells by decreasing intracellular cisplatin accumulation. Cancer Lett 329(2):189–196. https://doi.org/10.1016/j.canlet.2012.10.030
112. Liu M, Li CM, Chen ZF et al (2014) Celecoxib regulates apoptosis and autophagy via the PI3K/Akt signaling pathway in SGC-7901 gastric cancer cells. Int J Mol Med 33(6):1451–1458. https://doi.org/10.3892/ijmm.2014.1713

113. Meng C, Lu Z, Fang M et al (2014) Effect of celecoxib combined with chemotherapy drug on malignant biological behaviors of gastric cancer. Int J Clin Exp Pathol 7(11):7622–7632
114. Xu HB, Shen FM, Lv QZ (2015) Celecoxib enhanced the cytotoxic effect of cisplatin in drug-resistant human gastric cancer cells by inhibition of cyclooxygenase-2. Eur J Pharmacol 769: 1–7. https://doi.org/10.1016/j.ejphar.2015.09.025
115. Zhang L, Tong Y, Zhang X et al (2015) Arsenic sulfide combined with JQ1, chemotherapy agents, or celecoxib inhibit gastric and colon cancer cell growth. Drug Des Devel Ther 9: 5851–5862. https://doi.org/10.2147/DDDT.S92943
116. Zhang XQ, Sun XE, Liu WD et al (2015) Synergic effect between 5-fluorouracil and celecoxib on hypoxic gastric cancer cells. Mol Med Rep 11(2):1160–1166. https://doi.org/10.3892/mmr.2014.2783
117. Zhang XQ, Zhang HM, Sun XE et al (2015) Inhibitory effects and mechanism of 5-fluorouracil combined with celecoxib on human gastric cancer xenografts in nude mice. Exp Ther Med 9(1):105–111. https://doi.org/10.3892/etm.2014.2077
118. Xu HB, Shen FM, Lv QZ (2016) Celecoxib enhanced the cytotoxic effect of cisplatin in chemo-resistant gastric cancer xenograft mouse models through a cyclooxygenase-2-dependent manner. Eur J Pharmacol 776:1–8. https://doi.org/10.1016/j.ejphar.2016.02.035
119. Li Q, Peng J, Liu T et al (2017) Effects of celecoxib on cell apoptosis and Fas, FasL and Bcl-2 expression in a BGC-823 human gastric cancer cell line. Exp Ther Med 14(3):1935–1940. https://doi.org/10.3892/etm.2017.4769
120. Lin XM, Li S, Zhou C et al (2019) Cisplatin induces chemoresistance through the PTGS2-mediated anti-apoptosis in gastric cancer. Int J Biochem Cell Biol 116:105610. https://doi.org/10.1016/j.biocel.2019.105610
121. Choi SM, Cho YS, Park G et al (2021) Celecoxib induces apoptosis through Akt inhibition in 5-fluorouracil-resistant gastric cancer cells. Toxicol Res 37(1):25–33. https://doi.org/10.1007/s43188-020-00044-3
122. Badalanloo K, Naji T, Ahmadi R (2022) Cytotoxic and apoptotic effects of celecoxib and topotecan on AGS and HEK 293 cell lines. J Gastrointest Cancer 53(1):99–104. https://doi.org/10.1007/s12029-020-00434-8
123. Huang MT, Chen ZX, Wei B et al (2007) Preoperative growth inhibition of human gastric adenocarcinoma treated with a combination of celecoxib and octreotide. Acta Pharmacol Sin 28(11):1842–1850. https://doi.org/10.1111/j.1745-7254.2007.00652.x
124. Zhou Y, Ran J, Tang C et al (2007) Effect of celecoxib on E-cadherin, VEGF, microvessel density and apoptosis in gastric cancer. Cancer Biol Ther 6(2):269–275. https://doi.org/10.4161/cbt.6.2.3629
125. Han X, Li H, Su L et al (2014) Effect of celecoxib plus standard chemotherapy on serum levels of vascular endothelial growth factor and cyclooxygenase-2 in patients with gastric cancer. Biomed Rep 2(2):183–187. https://doi.org/10.3892/br.2013.209
126. Guo Q, Liu X, Lu L et al (2017) Comprehensive evaluation of clinical efficacy and safety of celecoxib combined with chemotherapy in management of gastric cancer. Medicine (Baltimore) 96(51):e8857. https://doi.org/10.1097/MD.0000000000008857
127. Guo Q, Li Q, Wang J et al (2019) A comprehensive evaluation of clinical efficacy and safety of celecoxib in combination with chemotherapy in metastatic or postoperative recurrent gastric cancer patients: a preliminary, three-center, clinical trial study. Medicine (Baltimore) 98(27): e16234. https://doi.org/10.1097/MD.0000000000016234
128. Brew T, Bougen-Zhukov N, Mitchell W et al (2021) Loss of E-cadherin leads to druggable vulnerabilities in sphingolipid metabolism and vesicle trafficking. Cancers 14(1). https://doi.org/10.3390/cancers14010102
129. Yan H, Sun Y, Wu Q et al (2019) PELP1 suppression inhibits gastric cancer through downregulation of c-Src-PI3K-ERK pathway. Front Oncol 9:1423. https://doi.org/10.3389/fonc.2019.01423

130. Hahm KB, Park IS, Kim HC et al (1996) Comparison of antiproliferative effects of 1-histamine-2 receptor antagonists, cimetidine, ranitidine, and famotidine, in gastric cancer cells. Int J Immunopharmacol 18(6-7):393–399. https://doi.org/10.1016/s0192-0561(96)00044-6
131. Jiang CG, Liu FR, Yu M et al (2010) Cimetidine induces apoptosis in gastric cancer cells in vitro and inhibits tumor growth in vivo. Oncol Rep 23(3):693–700. https://doi.org/10.3892/or_00000686
132. Liu FR, Jiang CG, Li YS et al (2011) Cimetidine inhibits the adhesion of gastric cancer cells expressing high levels of sialyl Lewis x in human vascular endothelial cells by blocking E-selectin expression. Int J Mol Med 27(4):537–544. https://doi.org/10.3892/ijmm.2011.618
133. Zhang L, Li Q, Xu J et al (2020) Cimetidine promotes STUB1-mediated degradation of tumoral FOXP3 by activating PI3K-Akt pathway in gastric cancer. Ann Transl Med 8(20):1304. https://doi.org/10.21037/atm-20-6070
134. Tonnesen H, Knigge U, Bulow S et al (1988) Effect of cimetidine on survival after gastric cancer. Lancet 2(8618):990–992. https://doi.org/10.1016/s0140-6736(88)90743-x
135. Langman MJ, Dunn JA, Whiting JL et al (1999) Prospective, double-blind, placebo-controlled randomized trial of cimetidine in gastric cancer. British Stomach Cancer Group. Br J Cancer 81(8):1356–1362. https://doi.org/10.1038/sj.bjc.6690457
136. Lin ZY, Kuo CH, Wu DC et al (2016) Anticancer effects of clinically acceptable colchicine concentrations on human gastric cancer cell lines. Kaohsiung J Med Sci 32(2):68–73. https://doi.org/10.1016/j.kjms.2015.12.006
137. Zhang T, Chen W, Jiang X et al (2019) Anticancer effects and underlying mechanism of Colchicine on human gastric cancer cell lines in vitro and in vivo. Biosci Rep 39(1). https://doi.org/10.1042/BSR20181802
138. Piontek M, Porschen R (1994) Growth inhibition of human gastrointestinal cancer cells by cyclosporin A. J Cancer Res Clin Oncol 120(12):695–699. https://doi.org/10.1007/BF01194265
139. Morisaki T, Matsunaga H, Beppu K et al (2000) A combination of cyclosporin-A (CsA) and interferon-gamma (INF-gamma) induces apoptosis in human gastric carcinoma cells. Anticancer Res 20(5B):3363–3373
140. Carl-McGrath S, Ebert MP, Lendeckel U et al (2007) Expression of the local angiotensin II system in gastric cancer may facilitate lymphatic invasion and nodal spread. Cancer Biol Ther 6(8):1218–1226. https://doi.org/10.4161/cbt.6.8.4412
141. Choi JH, Kim JS, Won YW et al (2016) The potential of deferasirox as a novel therapeutic modality in gastric cancer. World J Surg Oncol 14:77. https://doi.org/10.1186/s12957-016-0829-1
142. Kim JL, Lee DH, Na YJ et al (2016) Iron chelator-induced apoptosis via the ER stress pathway in gastric cancer cells. Tumour Biol 37(7):9709–9719. https://doi.org/10.1007/s13277-016-4878-4
143. Fujii T, Katoh M, Ootsubo M et al (2022) Cardiac glycosides stimulate endocytosis of GLUT1 via intracellular Na(+), K(+)-ATPase alpha3-isoform in human cancer cells. J Cell Physiol. https://doi.org/10.1002/jcp.30762
144. Sakaguchi Y, Maehara Y, Emi Y et al (1991) Dipyridamole combination chemotherapy can be used safely in treating gastric cancer patients. Anti-Cancer Drugs 2(2):139–143. https://doi.org/10.1097/00001813-199104000-00003
145. Wang IH, Huang TT, Chen JL et al (2020) Mevalonate pathway enzyme HMGCS1 contributes to gastric cancer progression. Cancers 12(5). https://doi.org/10.3390/cancers12051088
146. Kohnoe S, Maehara Y, Takahashi I et al (1998) Treatment of advanced gastric cancer with 5-fluorouracil and cisplatin in combination with dipyridamole. Int J Oncol 13(6):1203–1206. https://doi.org/10.3892/ijo.13.6.1203
147. Zhang J, Pu K, Bai S et al (2020) The anti-alcohol dependency drug disulfiram inhibits the viability and progression of gastric cancer cells by regulating the Wnt and NF-kappaB pathways. J Int Med Res 48(6):300060520925996. https://doi.org/10.1177/0300060520925996

148. Wang L, Chai X, Wan R et al (2020) Disulfiram Chelated With Copper Inhibits the Growth of Gastric Cancer Cells by Modulating Stress Response and Wnt/beta-catenin Signaling. Front Oncol 10:595718. https://doi.org/10.3389/fonc.2020.595718
149. Du C, Guan X, Liu Y et al (2022) Disulfiram/copper induces antitumor activity against gastric cancer cells in vitro and in vivo by inhibiting S6K1 and c-Myc. Cancer Chemother Pharmacol 89(4):451–458. https://doi.org/10.1007/s00280-022-04398-3
150. Liu Y, Guan X, Wang M et al (2022) Disulfiram/Copper induces antitumor activity against gastric cancer via the ROS/MAPK and NPL4 pathways. Bioengineered 13(3):6579–6589. https://doi.org/10.1080/21655979.2022.2038434
151. Pandian J, Panneerpandian P, Devanandan HJ et al (2020) Identification of the targeted therapeutic potential of doxycycline for a subset of gastric cancer patients. Ann N Y Acad Sci 1467(1):94–111. https://doi.org/10.1111/nyas.14288
152. Tang C, Yang L, Jiang X et al (2014) Antibiotic drug tigecycline inhibited cell proliferation and induced autophagy in gastric cancer cells. Biochem Biophys Res Commun 446(1): 105–112. https://doi.org/10.1016/j.bbrc.2014.02.043
153. Barranco SC, Ford PJ, Townsend CM Jr (1986) Heterogeneous survival responses of human gastric cancer clones to alpha difluoromethylornithine in vitro. Investig New Drugs 4(4): 337–345. https://doi.org/10.1007/BF00173506
154. Barranco SC, Ford PJ, Townsend CM Jr (1986) Cell cycle kinetics responses of human stomach cancer cells to reduction in polyamine levels by treatment with alpha difluoromethylornithine in vitro. Investig New Drugs 4(4):347–357. https://doi.org/10.1007/BF00173507
155. Fujimoto S, Igarashi K, Shrestha RD et al (1986) Combined therapy of polyamine antimetabolites and antitumor drugs for human gastric cancer xenotransplanted into nude mice. Jpn J Surg 16(2):133–139. https://doi.org/10.1007/BF02471083
156. Fujimoto S, Shrestha RD, Ohta M et al (1987) Enhanced antitumor efficacy with a combination of hyperthermochemotherapy and thermosensitization with polyamine antimetabolites in nude mice. Jpn J Surg 17(2):110–117. https://doi.org/10.1007/BF02470650
157. Shrestha RD, Fujimoto S, Okui K (1987) Contradictory antitumor efficacies produced by the combination of DNA attacking drugs and polyamine antimetabolites. Jpn J Surg 17(4): 263–268. https://doi.org/10.1007/BF02470698
158. Upp JR Jr, Beauchamp RD, Townsend CM Jr et al (1988) Inhibition of human gastric adenocarcinoma xenograft growth in nude mice by alpha-difluoromethylornithine. Cancer Res 48(11):3265–3269
159. Barranco SC, Townsend CM Jr, Ho BY et al (1990) Schedule dependent potentiation of antitumor drug effects by alpha-difluoromethylornithine in human gastric carcinoma cells in vitro. Investig New Drugs 8(Suppl 1):S9–S18. https://doi.org/10.1007/BF00171979
160. Kubota S (1992) Synergistic antiproliferative activity of human fibroblast interferon in combination with alpha-difluoromethylornithine against human gastric cancer cells in vitro. Cancer 69(10):2395–2399. https://doi.org/10.1002/1097-0142(19920515)69:10<2395::aid-cncr2820691002>3.0.co;2-h
161. Shrestha RD, Fujimoto S, Okui K (1992) A novel anticancer treatment for xenoplanted human gastric cancer using polyamine antimetabolites in a low polyamine diet. Surg Today 22(2): 137–142. https://doi.org/10.1007/BF00311338
162. Takahashi Y, Mai M, Nishioka K (2000) alpha-difluoromethylornithine induces apoptosis as well as anti-angiogenesis in the inhibition of tumor growth and metastasis in a human gastric cancer model. Int J Cancer 85(2):243–247
163. Chen M, Lu J, Wei W et al (2018) Effects of proton pump inhibitors on reversing multidrug resistance via downregulating V-ATPases/PI3K/Akt/mTOR/HIF-1alpha signaling pathway through TSC1/2 complex and Rheb in human gastric adenocarcinoma cells in vitro and in vivo. Onco Targets Ther 11:6705–6722. https://doi.org/10.2147/OTT.S161198

164. Xu Q, Jia X, Wu Q et al (2020) Esomeprazole affects the proliferation, metastasis, apoptosis and chemosensitivity of gastric cancer cells by regulating lncRNA/circRNA-miRNA-mRNA ceRNA networks. Oncol Lett 20(6):329. https://doi.org/10.3892/ol.2020.12193
165. Du J, Xu Q, Zhao H et al (2022) PI3K inhibitor 3-MA promotes the antiproliferative activity of esomeprazole in gastric cancer cells by downregulating EGFR via the PI3K/FOXO3a pathway. Biomed Pharmacother 148:112665. https://doi.org/10.1016/j.biopha.2022.112665
166. Hinsenkamp I, Schulz S, Roscher M et al (2016) Inhibition of rho-associated kinase 1/2 attenuates tumor growth in murine gastric cancer. Neoplasia 18(8):500–511. https://doi.org/10.1016/j.neo.2016.07.002
167. Chen L, Peng J, Wang Y et al (2020) Fenofibrate-induced mitochondrial dysfunction and metabolic reprogramming reversal: the anti-tumor effects in gastric carcinoma cells mediated by the PPAR pathway. Am J Transl Res 12(2):428–446
168. Zheng T, Meng X, Wang J et al (2010) PTEN- and p53-mediated apoptosis and cell cycle arrest by FTY720 in gastric cancer cells and nude mice. J Cell Biochem 111(1):218–228. https://doi.org/10.1002/jcb.22691
169. Khing TM, Po WW, Sohn UD (2019) Fluoxetine enhances anti-tumor activity of paclitaxel in gastric adenocarcinoma cells by triggering apoptosis and necroptosis. Anticancer Res 39(11):6155–6163. https://doi.org/10.21873/anticanres.13823
170. Khin PP, Po WW, Thein W et al (2020) Apoptotic effect of fluoxetine through the endoplasmic reticulum stress pathway in the human gastric cancer cell line AGS. Naunyn Schmiedeberg's Arch Pharmacol 393(4):537–549. https://doi.org/10.1007/s00210-019-01739-7
171. Po WW, Thein W, Khin PP et al (2020) Fluoxetine simultaneously induces both apoptosis and autophagy in human gastric adenocarcinoma cells. Biomol Ther (Seoul) 28(2):202–210. https://doi.org/10.4062/biomolther.2019.103
172. Qian X, Li J, Ding J et al (2008) Glibenclamide exerts an antitumor activity through reactive oxygen species-c-jun NH2-terminal kinase pathway in human gastric cancer cell line MGC-803. Biochem Pharmacol 76(12):1705–1715. https://doi.org/10.1016/j.bcp.2008.09.009
173. Wang W, Liu L, Zhou Y et al (2019) Hydroxychloroquine enhances the antitumor effects of BC001 in gastric cancer. Int J Oncol 55(2):405–414. https://doi.org/10.3892/ijo.2019.4824
174. Bonelli P, Tuccillo FM, Calemma R et al (2011) Changes in the gene expression profile of gastric cancer cells in response to ibuprofen: a gene pathway analysis. Pharmacogenomics J 11(6):412–428. https://doi.org/10.1038/tpj.2010.55
175. Akrami H, Moradi B, Borzabadi Farahani D et al (2018) Ibuprofen reduces cell proliferation through inhibiting Wnt/beta catenin signaling pathway in gastric cancer stem cells. Cell Biol Int 42(8):949–958. https://doi.org/10.1002/cbin.10959
176. Ding XW, Luo HS, Jin X et al (2007) Aberrant expression of Eag1 potassium channels in gastric cancer patients and cell lines. Med Oncol 24(3):345–350. https://doi.org/10.1007/s12032-007-0015-y
177. Sawaoka H, Kawano S, Tsuji S et al (1998) Effects of NSAIDs on proliferation of gastric cancer cells in vitro: possible implication of cyclooxygenase-2 in cancer development. J Clin Gastroenterol 27(Suppl 1):S47–S52. https://doi.org/10.1097/00004836-199800001-00009
178. Chiou SK, Hoa N, Hodges A et al (2014) Indomethacin promotes apoptosis in gastric cancer cells through concomitant degradation of Survivin and Aurora B kinase proteins. Apoptosis 19(9):1378–1388. https://doi.org/10.1007/s10495-014-1002-3
179. Ito H, Matsui H, Hirayama A et al (2016) Reactive oxygen species induced by non-steroidal anti-inflammatory drugs enhance the effects of photodynamic therapy in gastric cancer cells. J Clin Biochem Nutr 58(3):180–185. https://doi.org/10.3164/jcbn.15-124
180. Vallecillo-Hernandez J, Barrachina MD, Ortiz-Masia D et al (2018) Indomethacin disrupts autophagic flux by inducing lysosomal dysfunction in gastric cancer cells and increases their sensitivity to cytotoxic drugs. Sci Rep 8(1):3593. https://doi.org/10.1038/s41598-018-21455-1

181. Mazumder S, De R, Debsharma S et al (2019) Indomethacin impairs mitochondrial dynamics by activating the PKCzeta-p38-DRP1 pathway and inducing apoptosis in gastric cancer and normal mucosal cells. J Biol Chem 294(20):8238–8258. https://doi.org/10.1074/jbc.RA118.004415
182. Hara M, Nagasaki T, Shiga K et al (2016) Suppression of cancer-associated fibroblasts and endothelial cells by itraconazole in bevacizumab-resistant gastrointestinal cancer. Anticancer Res 36(1):169–177
183. Hu Q, Hou YC, Huang J et al (2017) Itraconazole induces apoptosis and cell cycle arrest via inhibiting Hedgehog signaling in gastric cancer cells. J Exp Clin Cancer Res 36(1):50. https://doi.org/10.1186/s13046-017-0526-0
184. Lan K, Yan R, Zhu K et al (2018) Itraconazole inhibits the proliferation of gastric cancer cells in vitro and improves patient survival. Oncol Lett 16(3):3651–3657. https://doi.org/10.3892/ol.2018.9072
185. Zhao S, Shao L, Wang Y et al (2020) Ketamine exhibits anti-gastric cancer activity via induction of apoptosis and attenuation of PI3K/Akt/mTOR. Arch Med Sci 16(5):1140–1149. https://doi.org/10.5114/aoms.2019.85146
186. Miwa H, Ono F, Moriyama M et al (1980) Immunochemotherapy of gastric cancer with levamisole. Acta Med Okayama 34(4):275–281. https://doi.org/10.18926/AMO/30518
187. Hattori T, Niimoto M, Toge T et al (1983) Effects of Levamisole in adjuvant immunochemotherapy for gastric cancer; a prospective randomized controlled study. Jpn J Surg 13(6):480–485. https://doi.org/10.1007/BF02469490
188. Niimoto M, Hattori T, Ito I et al (1984) Levamisole in postoperative adjuvant immunochemotherapy for gastric cancer. A randomized controlled study of the MMC + Tegafur regimen with or without levamisole. Report I. Cancer Immunol Immunother 18(1):13–18. https://doi.org/10.1007/BF00205393
189. Group TIGTS (1988) Adjuvant treatments following curative resection for gastric cancer. The Italian Gastrointestinal Tumor Study Group. Br J Surg 75(11):1100–1104. https://doi.org/10.1002/bjs.1800751117
190. Choi JS, Lee KH, Ahn MJ et al (1997) A randomized trial comparing cisplatin plus 5-fluorouracil with or without levamisole in operable gastric cancer. Korean J Intern Med 12(2):155–162. https://doi.org/10.3904/kjim.1997.12.2.155
191. Burch PA, Keppen MD, Schroeder G et al (1999) North Central Cancer Treatment Group Phase II study of 5-fluorouracil and high-dose levamisole for gastric and gastroesophageal cancer using survival as the primary endpoint of efficacy. Am J Clin Oncol 22(5):505–508. https://doi.org/10.1097/00000421-199910000-00017
192. Yang W, Cai J, Zhang H et al (2018) Effects of lidocaine and ropivacaine on gastric cancer cells through down-regulation of ERK1/2 phosphorylation in vitro. Anticancer Res 38(12):6729–6735. https://doi.org/10.21873/anticanres.13042
193. Ye L, Zhang Y, Chen YJ et al (2019) Anti-tumor effects of lidocaine on human gastric cancer cells in vitro. Bratisl Lek Listy 120(3):212–217. https://doi.org/10.4149/BLL_2019_036
194. Sui H, Lou A, Li Z et al (2019) Lidocaine inhibits growth, migration and invasion of gastric carcinoma cells by up-regulation of miR-145. BMC Cancer 19(1):233. https://doi.org/10.1186/s12885-019-5431-9
195. Zhang X, Gu G, Li X et al (2020) Lidocaine alleviates cisplatin resistance and inhibits migration of MGC-803/DDP cells through decreasing miR-10b. Cell Cycle 19(19):2530–2537. https://doi.org/10.1080/15384101.2020.1809914
196. Zeng W, Xing ZT, Tan MY et al (2021) Lidocaine suppresses the malignant behavior of gastric cancer cells via the c-Met/c-Src pathway. Exp Ther Med 21(5):424. https://doi.org/10.3892/etm.2021.9868
197. Guan E, Liu H, Xu N (2021) Lidocaine suppresses gastric cancer development through Circ_ANO5/miR-21-5p/LIFR Axis. Dig Dis Sci. 67(6):2244–2256. https://doi.org/10.1007/s10620-021-07055-6

198. Huang MM, Guo AB, Sun JF et al (2014) Angiotensin II promotes the progression of human gastric cancer. Mol Med Rep 9(3):1056–1060. https://doi.org/10.3892/mmr.2014.1891
199. Rha SY, Noh SH, Kim TS et al (1999) Modulation of biological phenotypes for tumor growth and metastasis by target-specific biological inhibitors in gastric cancer. Int J Mol Med 4(2): 203–212. https://doi.org/10.3892/ijmm.4.2.203
200. Kang WK, Lee I, Park C (2005) Characterization of RhoA-mediated chemoresistance in gastric cancer cells. Cancer Res Treat 37(4):251–256. https://doi.org/10.4143/crt.2005.37.4.251
201. Gibot L, Follet J, Metges JP et al (2009) Human caspase 7 is positively controlled by SREBP-1 and SREBP-2. Biochem J 420(3):473–483. https://doi.org/10.1042/BJ20082057
202. Follet J, Remy L, Hesry V et al (2011) Adaptation to statins restricts human tumour growth in Nude mice. BMC Cancer 11:491. https://doi.org/10.1186/1471-2407-11-491
203. Follet J, Corcos L, Baffet G et al (2012) The association of statins and taxanes: an efficient combination trigger of cancer cell apoptosis. Br J Cancer 106(4):685–692. https://doi.org/10.1038/bjc.2012.6
204. Cheng-Qian Y, Xin-Jing W, Wei X et al (2014) Lovastatin inhibited the growth of gastric cancer cells. Hepato-Gastroenterology 61(129):1–4
205. Lin L, Liu Y, Pan C et al (2019) Gastric cancer cells escape metabolic stress via the DLC3/MACC1 axis. Theranostics 9(7):2100–2114. https://doi.org/10.7150/thno.29538
206. Zhang L, Kang W, Lu X et al (2019) Weighted gene co-expression network analysis and connectivity map identifies lovastatin as a treatment option of gastric cancer by inhibiting HDAC2. Gene 681:15–25. https://doi.org/10.1016/j.gene.2018.09.040
207. Pereira PMR, Mandleywala K, Ragupathi A et al (2019) Temporal modulation of HER2 membrane availability increases pertuzumab uptake and pretargeted molecular imaging of gastric tumors. J Nucl Med 60(11):1569–1578. https://doi.org/10.2967/jnumed.119.225813
208. Alzeeb G, Arzur D, Trichet V et al (2022) Gastric cancer cell death analyzed by live cell imaging of spheroids. Sci Rep 12(1):1488. https://doi.org/10.1038/s41598-022-05426-1
209. Kim WS, Kim MM, Choi HJ et al (2001) Phase II study of high-dose lovastatin in patients with advanced gastric adenocarcinoma. Investig New Drugs 19(1):81–83. https://doi.org/10.1023/a:1006481423298
210. Yamakawa N, Suemasu S, Kimoto A et al (2010) Low direct cytotoxicity of loxoprofen on gastric mucosal cells. Biol Pharm Bull 33(3):398–403. https://doi.org/10.1248/bpb.33.398
211. Mencarelli A, Graziosi L, Renga B et al (2013) CCR5 antagonism by maraviroc reduces the potential for gastric cancer cell dissemination. Transl Oncol 6(6):784–793. https://doi.org/10.1593/tlo.13499
212. Pinto LC, Soares BM, Pinheiro Jde J et al (2015) The anthelmintic drug mebendazole inhibits growth, migration and invasion in gastric cancer cell model. Toxicol in Vitro 29(8): 2038–2044. https://doi.org/10.1016/j.tiv.2015.08.007
213. Celestino Pinto L, de Fatima Aquino Moreira-Nunes C, Soares BM et al (2017) Mebendazole, an antiparasitic drug, inhibits drug transporters expression in preclinical model of gastric peritoneal carcinomatosis. Toxicol in Vitro 43:87–91. https://doi.org/10.1016/j.tiv.2017.06.007
214. Pinto LC, Mesquita FP, Soares BM et al (2019) Mebendazole induces apoptosis via C-MYC inactivation in malignant ascites cell line (AGP01). Toxicol in Vitro 60:305–312. https://doi.org/10.1016/j.tiv.2019.06.010
215. Liu Y, Chen S, Xue R et al (2016) Mefloquine effectively targets gastric cancer cells through phosphatase-dependent inhibition of PI3K/Akt/mTOR signaling pathway. Biochem Biophys Res Commun 470(2):350–355. https://doi.org/10.1016/j.bbrc.2016.01.046
216. Zhang S, Zuo L, Gui S et al (2012) Induction of cell differentiation and promotion of endocan gene expression in stomach cancer by melatonin. Mol Biol Rep 39(3):2843–2849. https://doi.org/10.1007/s11033-011-1043-4

217. Zhang S, Qi Y, Zhang H et al (2013) Melatonin inhibits cell growth and migration, but promotes apoptosis in gastric cancer cell line, SGC7901. Biotech Histochem 88(6):281–289. https://doi.org/10.3109/10520295.2013.769633
218. Li W, Fan M, Chen Y et al (2015) Melatonin induces cell apoptosis in AGS cells through the activation of JNK and P38 MAPK and the suppression of nuclear factor-kappa B: a novel therapeutic implication for gastric cancer. Cell Physiol Biochem 37(6):2323–2338. https://doi.org/10.1159/000438587
219. Wu SM, Lin WY, Shen CC et al (2016) Melatonin set out to ER stress signaling thwarts epithelial mesenchymal transition and peritoneal dissemination via calpain-mediated C/EBPbeta and NFkappaB cleavage. J Pineal Res 60(2):142–154. https://doi.org/10.1111/jpi.12295
220. Wang RX, Liu H, Xu L et al (2016) Melatonin downregulates nuclear receptor RZR/RORgamma expression causing growth-inhibitory and anti-angiogenesis activity in human gastric cancer cells in vitro and in vivo. Oncol Lett 12(2):897–903. https://doi.org/10.3892/ol.2016.4729
221. Li W, Wang Z, Chen Y et al (2017) Melatonin treatment induces apoptosis through regulating the nuclear factor-kappaB and mitogen-activated protein kinase signaling pathways in human gastric cancer SGC7901 cells. Oncol Lett 13(4):2737–2744. https://doi.org/10.3892/ol.2017.5785
222. Wang X, Wang B, Xie J et al (2018) Melatonin inhibits epithelial to mesenchymal transition in gastric cancer cells via attenuation of IL1 beta/NFkappaB/MMP2/MMP9 signaling. Int J Mol Med 42(4):2221–2228. https://doi.org/10.3892/ijmm.2018.3788
223. Wei X, Qi Y, Jia N et al (2018) Hyperbaric oxygen treatment sensitizes gastric cancer cells to melatonin-induced apoptosis through multiple pathways. J Cell Biochem 119(8):6723–6731. https://doi.org/10.1002/jcb.26864
224. Zhu C, Huang Q, Zhu H (2018) Melatonin inhibits the proliferation of gastric cancer cells through regulating the miR-16-5p-Smad3 pathway. DNA Cell Biol 37(3):244–252. https://doi.org/10.1089/dna.2017.4040
225. Song J, Ma SJ, Luo JH et al (2018) Melatonin induces the apoptosis and inhibits the proliferation of human gastric cancer cells via blockade of the AKT/MDM2 pathway. Oncol Rep 39(4):1975–1983. https://doi.org/10.3892/or.2018.6282
226. Zheng Y, Tu J, Wang X et al (2019) The therapeutic effect of melatonin on GC by inducing cell apoptosis and autophagy induced by endoplasmic reticulum stress. Onco Targets Ther 12:10187–10198. https://doi.org/10.2147/OTT.S226140
227. Wei X, Chen S, Xu Z et al (2019) Melatonin inhibits the migration of human gastric carcinoma cells at least in part by remodeling tight junction. J Cell Biochem 120(6):9781–9786. https://doi.org/10.1002/jcb.28258
228. Song J, Ma SJ, Luo JH et al (2019) Downregulation of AKT and MDM2, melatonin induces apoptosis in AGS and MGC803 cells. Anat Rec (Hoboken) 302(9):1544–1551. https://doi.org/10.1002/ar.24101
229. Wang X, Wang B, Zhan W et al (2019) Melatonin inhibits lung metastasis of gastric cancer in vivo. Biomed Pharmacother 117:109018. https://doi.org/10.1016/j.biopha.2019.109018
230. Wang R, Liu H, Song J et al (2021) Activity of melatonin against gastric cancer growth in a chick embryo tumor xenograft model. Cancer Manag Res 13:8803–8808. https://doi.org/10.2147/CMAR.S329728
231. Huang Y, Yuan K, Tang M et al (2021) Melatonin inhibiting the survival of human gastric cancer cells under ER stress involving autophagy and Ras-Raf-MAPK signalling. J Cell Mol Med 25(3):1480–1492. https://doi.org/10.1111/jcmm.16237
232. Liu D, Shi K, Fu M et al (2021) Melatonin indirectly decreases gastric cancer cell proliferation and invasion via effects on cancer-associated fibroblasts. Life Sci 277:119497. https://doi.org/10.1016/j.lfs.2021.119497

233. Li W, Hu C, Zhong X et al (2022) Melatonin induces AGS gastric cancer cell apoptosis via regulating PERK/eIF2alpha and HSF1/NF-kappaB signaling pathway. Ann Clin Lab Sci 52(1):40–47
234. Lissoni P, Barni S, Tancini G et al (1993) Immunotherapy with subcutaneous low-dose interleukin-2 and the pineal indole melatonin as a new effective therapy in advanced cancers of the digestive tract. Br J Cancer 67(6):1404–1407. https://doi.org/10.1038/bjc.1993.260
235. Lissoni P, Brivio F, Ardizzoia A et al (1993) Subcutaneous therapy with low-dose interleukin-2 plus the neurohormone melatonin in metastatic gastric cancer patients with low performance status. Tumori 79(6):401–404
236. Lissoni P (2007) Biochemotherapy with standard chemotherapies plus the pineal hormone melatonin in the treatment of advanced solid neoplasms. Pathol Biol (Paris) 55(3–4):201–204. https://doi.org/10.1016/j.patbio.2006.12.025
237. Kato K, Gong J, Iwama H et al (2012) The antidiabetic drug metformin inhibits gastric cancer cell proliferation in vitro and in vivo. Mol Cancer Ther 11(3):549–560. https://doi.org/10.1158/1535-7163.MCT-11-0594
238. Fang W, Cui H, Yu D et al (2014) Increased expression of phospho-acetyl-CoA carboxylase protein is an independent prognostic factor for human gastric cancer without lymph node metastasis. Med Oncol 31(7):15. https://doi.org/10.1007/s12032-014-0015-7
239. Lesan V, Ghaffari SH, Salaramoli J et al (2014) Evaluation of antagonistic effects of metformin with Cisplatin in gastric cancer cells. Int J Hematol Oncol Stem Cell Res 8(3):12–19
240. Chen G, Feng W, Zhang S et al (2015) Metformin inhibits gastric cancer via the inhibition of HIF1alpha/PKM2 signaling. Am J Cancer Res 5(4):1423–1434
241. Yu G, Fang W, Xia T et al (2015) Metformin potentiates rapamycin and cisplatin in gastric cancer in mice. Oncotarget 6(14):12748–12762. https://doi.org/10.18632/oncotarget.3327
242. Han G, Gong H, Wang Y et al (2015) AMPK/mTOR-mediated inhibition of survivin partly contributes to metformin-induced apoptosis in human gastric cancer cell. Cancer Biol Ther 16(1):77–87. https://doi.org/10.4161/15384047.2014.987021
243. Huang D, He X, Zou J et al (2016) Negative regulation of Bmi-1 by AMPK and implication in cancer progression. Oncotarget 7(5):6188–6200. https://doi.org/10.18632/oncotarget.6748
244. Courtois S, Duran RV, Giraud J et al (2017) Metformin targets gastric cancer stem cells. Eur J Cancer 84:193–201. https://doi.org/10.1016/j.ejca.2017.07.020
245. Song Z, Wei B, Lu C et al (2017) Metformin suppresses the expression of Sonic hedgehog in gastric cancer cells. Mol Med Rep 15(4):1909–1915. https://doi.org/10.3892/mmr.2017.6205
246. Zhuang S, Jian Y, Sun Y (2017) Metformin inhibits N-Methyl-N-nitrosourea induced gastric tumorigenesis in db/db mice. Exp Clin Endocrinol Diabetes 125(6):392–399. https://doi.org/10.1055/s-0043-100118
247. Wu X (2017) Effect of metformin combined with chemotherapeutic agents on gastric cancer cell line AGS. Pak J Pharm Sci 30(5(Special)):1833–1836
248. Valaee S, Yaghoobi MM, Shamsara M (2017) Metformin inhibits gastric cancer cells metastatic traits through suppression of epithelial-mesenchymal transition in a glucose-independent manner. PLoS One 12(3):e0174486. https://doi.org/10.1371/journal.pone.0174486
249. Li W, Wong CC, Zhang X et al (2018) CAB39L elicited an anti-Warburg effect via a LKB1-AMPK-PGC1alpha axis to inhibit gastric tumorigenesis. Oncogene 37(50):6383–6398. https://doi.org/10.1038/s41388-018-0402-1
250. Sekino N, Kano M, Matsumoto Y et al (2018) The antitumor effects of metformin on gastric cancer in vitro and on peritoneal metastasis. Anticancer Res 38(11):6263–6269. https://doi.org/10.21873/anticanres.12982
251. Tseng HH, Chen YZ, Chou NH et al (2021) Metformin inhibits gastric cancer cell proliferation by regulation of a novel Loc100506691-CHAC1 axis. Mol Ther Oncol 22:180–194. https://doi.org/10.1016/j.omto.2021.08.006
252. Bae WJ, Ahn JM, Byeon HE et al (2019) PTPRD-inactivation-induced CXCL8 promotes angiogenesis and metastasis in gastric cancer and is inhibited by metformin. J Exp Clin Cancer Res 38(1):484. https://doi.org/10.1186/s13046-019-1469-4

253. Lu CC, Chiang JH, Tsai FJ et al (2019) Metformin triggers the intrinsic apoptotic response in human AGS gastric adenocarcinoma cells by activating AMPK and suppressing mTOR/AKT signaling. Int J Oncol 54(4):1271–1281. https://doi.org/10.3892/ijo.2019.4704
254. Li P, Tong L, Song Y et al (2019) Long noncoding RNA H19 participates in metformin-mediated inhibition of gastric cancer cell invasion. J Cell Physiol 234(4):4515–4527. https://doi.org/10.1002/jcp.27269
255. Chen G, Yu C, Tang Z et al (2019) Metformin suppresses gastric cancer progression through calmodulin-like protein 3 secreted from tumor-associated fibroblasts. Oncol Rep 41(1): 405–414. https://doi.org/10.3892/or.2018.6783
256. Chen Y, Gong W, Zhou Y et al (2020) Metformin up-regulated miR-107 expression and enhanced the inhibitory effect of miR-107 on gastric cancer growth. Transl Cancer Res 9(4): 2941–2950. https://doi.org/10.21037/tcr.2020.03.25
257. Liu S, Yue C, Chen H et al (2020) Metformin promotes beclin1-dependent autophagy to inhibit the progression of gastric cancer. Onco Targets Ther 13:4445–4455. https://doi.org/10.2147/OTT.S242298
258. Miao ZF, Adkins-Threats M, Burclaff JR et al (2020) A metformin-responsive metabolic pathway controls distinct steps in gastric progenitor fate decisions and maturation. Cell Stem Cell 26(6):910–25.e6. https://doi.org/10.1016/j.stem.2020.03.006
259. Valaee S, Shamsara M, Yaghoobi MM (2021) Metformin is a novel suppressor for vimentin in human gastric cancer cell line. Int J Mol Cell Med 10(3):200–206. https://doi.org/10.22088/IJMCM.BUMS.10.3.200
260. Wang WH, Chen SK, Huang HC et al (2021) Proteomic analysis reveals that metformin suppresses PSMD2, STIP1, and CAP1 for preventing gastric cancer AGS cell proliferation and migration. ACS Omega 6(22):14208–14219. https://doi.org/10.1021/acsomega.1c00894
261. Zou J, Li C, Jiang S et al (2021) AMPK inhibits Smad3-mediated autoinduction of TGF-beta1 in gastric cancer cells. J Cell Mol Med 25(6):2806–2815. https://doi.org/10.1111/jcmm.16308
262. Fatehi-Agdam M, Vatankhah MA, Panahizadeh R et al (2021) Efficacy of metformin and chemotherapeutic agents on the inhibition of colony formation and Shh/Gli1 pathway: metformin/docetaxel versus metformin/5-fluorouracil. Drug Res (Stuttg) 71(1):17–25. https://doi.org/10.1055/a-1248-9008
263. Deng T, Shen P, Li A et al (2021) CCDC65 as a new potential tumor suppressor induced by metformin inhibits activation of AKT1 via ubiquitination of ENO1 in gastric cancer. Theranostics 11(16):8112–8128. https://doi.org/10.7150/thno.54961
264. Wang J, Huang Q, Hu X et al (2022) Disrupting circadian rhythm via the PER1-HK2 Axis reverses trastuzumab resistance in gastric cancer. Cancer Res 82(8):1503–1517. https://doi.org/10.1158/0008-5472.CAN-21-1820
265. Huang F, Xiang Y, Li T et al (2022) Metformin and MiR-365 synergistically promote the apoptosis of gastric cancer cells via MiR-365-PTEN-AMPK axis. Pathol Res Pract 230: 153740. https://doi.org/10.1016/j.prp.2021.153740
266. Fujimoto S, Ohta M, Shrestha RD et al (1988) Enhancement of hyperthermochemotherapy for human gastric cancer in nude mice by thermosensitization with nitroimidazoles. Br J Cancer 58(1):42–45. https://doi.org/10.1038/bjc.1988.158
267. Skoropad VY, Berdov BA, Zagrebin VM (2003) Preoperative radiotherapy in combination with metronidazole for resectable gastric cancer: long-term results of a phase 2 study. Eur J Surg Oncol 29(2):166–170. https://doi.org/10.1053/ejso.2002.1324
268. Yu HE, Wang F, Yu F et al (2019) Suppression of fumarate hydratase activity increases the efficacy of cisplatin-mediated chemotherapy in gastric cancer. Cell Death Dis 10(6):413. https://doi.org/10.1038/s41419-019-1652-8
269. Li DQ, Pan LH, Shao ZM (2004) Inhibitory effects of mifepristone on the growth of human gastric cancer cell line MKN-45 in vitro and in vivo. Chin Med Sci J 19(4):237–242
270. Li DQ, Wang ZB, Bai J et al (2004) Reversal of multidrug resistance in drug-resistant human gastric cancer cell line SGC7901/VCR by antiprogestin drug mifepristone. World J Gastroenterol 10(12):1722–1725. https://doi.org/10.3748/wjg.v10.i12.1722

271. Nakamura A, Matsunaga W, Gotoh A (2018) Autophagy induced by naftopidil inhibits apoptosis of human gastric cancer cells. Anticancer Res 38(2):803–809. https://doi.org/10.21873/anticanres.12287
272. Treese C, Hartl K, Potzsch M et al (2022) S100A4 is a strong negative prognostic marker and potential therapeutic target in adenocarcinoma of the stomach and esophagus. Cells 11(6). https://doi.org/10.3390/cells11061056
273. Baoping Y, Guoyong H, Jieping Y et al (2004) Cyclooxygenase-2 inhibitor nimesulide suppresses telomerase activity by blocking Akt/PKB activation in gastric cancer cell line. Dig Dis Sci 49(6):948–953. https://doi.org/10.1023/b:ddas.0000034553.58554.ab
274. Liu M, Luo XJ, Liao F et al (2011) Noscapine induces mitochondria-mediated apoptosis in gastric cancer cells in vitro and in vivo. Cancer Chemother Pharmacol 67(3):605–612. https://doi.org/10.1007/s00280-010-1356-3
275. Gao S, Yu BP, Li Y et al (2003) Antiproliferative effect of octreotide on gastric cancer cells mediated by inhibition of Akt/PKB and telomerase. World J Gastroenterol 9(10):2362–2365. https://doi.org/10.3748/wjg.v9.i10.2362
276. Wang CH, Tang CW, Liu CL et al (2003) Inhibitory effect of octreotide on gastric cancer growth via MAPK pathway. World J Gastroenterol 9(9):1904–1908. https://doi.org/10.3748/wjg.v9.i9.1904
277. Tang C, Liu C, Zhou X et al (2004) Enhanced inhibitive effects of combination of rofecoxib and octreotide on the growth of human gastric cancer. Int J Cancer 112(3):470–474. https://doi.org/10.1002/ijc.20256
278. Hu C, Yi C, Hao Z et al (2004) The effect of somatostatin and SSTR3 on proliferation and apoptosis of gastric cancer cells. Cancer Biol Ther 3(8):726–730. https://doi.org/10.4161/cbt.3.8.962
279. El-Salhy M (2005) Effects of octreotide, galanin and serotonin on a human gastric cancer cell line. Oncol Rep 13(5):787–791. https://doi.org/10.3892/or.13.5.787
280. Wang L, Huang X, Chai Y et al (2017) Octreotide inhibits the proliferation of gastric cancer cells through P300-HAT activity and the interaction of ZAC and P300. Oncol Rep 37(4):2041–2048. https://doi.org/10.3892/or.2017.5451
281. Feng S, Qiu G, Yang L et al (2021) Omeprazole improves chemosensitivity of gastric cancer cells by m6A demethylase FTO-mediated activation of mTORC1 and DDIT3 up-regulation. Biosci Rep 41(1). https://doi.org/10.1042/BSR20200842
282. Dowling S, Cox J, Cenedella RJ (2009) Inhibition of fatty acid synthase by Orlistat accelerates gastric tumor cell apoptosis in culture and increases survival rates in gastric tumor bearing mice in vivo. Lipids 44(6):489–498. https://doi.org/10.1007/s11745-009-3298-2
283. Cao B, Deng H, Cui H et al (2021) Knockdown of PGM1 enhances anticancer effects of orlistat in gastric cancer under glucose deprivation. Cancer Cell Int 21(1):481. https://doi.org/10.1186/s12935-021-02193-3
284. Chen HY, Yang MD, Chou YC et al (2021) Ouabain Suppresses Cell Migration and Invasion in Human Gastric Cancer AGS Cells Through the Inhibition of MMP Signaling Pathways. Anticancer Res 41(9):4365–4375. https://doi.org/10.21873/anticanres.15241
285. Yeo M, Kim DK, Kim YB et al (2004) Selective induction of apoptosis with proton pump inhibitor in gastric cancer cells. Clin Cancer Res 10(24):8687–8696. https://doi.org/10.1158/1078-0432.CCR-04-1065
286. Huang S, Chen M, Ding X et al (2013) Proton pump inhibitor selectively suppresses proliferation and restores the chemosensitivity of gastric cancer cells by inhibiting STAT3 signaling pathway. Int Immunopharmacol 17(3):585–592. https://doi.org/10.1016/j.intimp.2013.07.021
287. Shen W, Zou X, Chen M et al (2013) Effect of pantoprazole on human gastric adenocarcinoma SGC7901 cells through regulation of phosphoLRP6 expression in Wnt/beta-catenin signaling. Oncol Rep 30(2):851–855. https://doi.org/10.3892/or.2013.2524
288. Shen Y, Chen M, Huang S et al (2016) Pantoprazole inhibits human gastric adenocarcinoma SGC-7901 cells by downregulating the expression of pyruvate kinase M2. Oncol Lett 11(1):717–722. https://doi.org/10.3892/ol.2015.3912

289. Feng S, Zheng Z, Feng L et al (2016) Proton pump inhibitor pantoprazole inhibits the proliferation, self-renewal and chemoresistance of gastric cancer stem cells via the EMT/betacatenin pathways. Oncol Rep 36(6):3207–3214. https://doi.org/10.3892/or.2016.5154
290. Koh JS, Joo MK, Park JJ et al (2018) Inhibition of STAT3 in gastric cancer: role of pantoprazole as SHP-1 inducer. Cell Biosci 8:50. https://doi.org/10.1186/s13578-018-0248-9
291. Zhang B, Ling T, Zhaxi P et al (2019) Proton pump inhibitor pantoprazole inhibits gastric cancer metastasis via suppression of telomerase reverse transcriptase gene expression. Cancer Lett 452:23–30. https://doi.org/10.1016/j.canlet.2019.03.029
292. Liu BH, Yuan TM, Huang CJ et al (2022) DNA repair proteins as the targets for paroxetine to induce cytotoxicity in gastric cancer cell AGS. Am J Cancer Res 12(4):1465–1483
293. Wang Y, Lu JH, Wang F et al (2020) Inhibition of fatty acid catabolism augments the efficacy of oxaliplatin-based chemotherapy in gastrointestinal cancers. Cancer Lett 473:74–89. https://doi.org/10.1016/j.canlet.2019.12.036
294. Takahashi N, Okumura T, Motomura W et al (1999) Activation of PPARgamma inhibits cell growth and induces apoptosis in human gastric cancer cells. FEBS Lett 455(1–2):135–139. https://doi.org/10.1016/s0014-5793(99)00871-6
295. Konings IR, van der Gaast A, van der Wijk LJ et al (2010) The addition of pravastatin to chemotherapy in advanced gastric carcinoma: a randomised phase II trial. Eur J Cancer 46(18):3200–3204. https://doi.org/10.1016/j.ejca.2010.07.036
296. Peng Z, Zhang Y (2016) Propofol inhibits proliferation and accelerates apoptosis of human gastric cancer cells by regulation of microRNA-451 and MMP-2 expression. Genet Mol Res 15(2). https://doi.org/10.4238/gmr.15027078
297. Yang C, Gao J, Yan N et al (2017) Propofol inhibits the growth and survival of gastric cancer cells in vitro through the upregulation of ING3. Oncol Rep 37(1):587–593. https://doi.org/10.3892/or.2016.5218
298. Liu F, Qiu F, Fu M et al (2020) Propofol reduces epithelial to mesenchymal transition, invasion and migration of gastric cancer cells through the MicroRNA-195-5p/snail axis. Med Sci Monit 26:e920981. https://doi.org/10.12659/MSM.920981
299. Sui H, Zhu C, Li Z et al (2020) Propofol suppresses gastric cancer tumorigenesis by modulating the circular RNAPVT1/miR1955p/E26 oncogene homolog 1 axis. Oncol Rep 44(4):1736–1746. https://doi.org/10.3892/or.2020.7725
300. Zhang YF, Li CS, Zhou Y et al (2020) Propofol facilitates cisplatin sensitivity via lncRNA MALAT1/miR-30e/ATG5 axis through suppressing autophagy in gastric cancer. Life Sci 244:117280. https://doi.org/10.1016/j.lfs.2020.117280
301. Liu YP, Qiu ZZ, Li XH et al (2021) Propofol induces ferroptosis and inhibits malignant phenotypes of gastric cancer cells by regulating miR-125b-5p/STAT3 axis. World J Gastrointest Oncol 13(12):2114–2128. https://doi.org/10.4251/wjgo.v13.i12.2114
302. Bai ZM, Li XF, Yang Y et al (2021) Propofol inhibited gastric cancer proliferation via the hsa-miR-328-3p/STAT3 pathway. Clin Transl Oncol 23(9):1866–1873. https://doi.org/10.1007/s12094-021-02595-9
303. Liu L, Dong T, Sheng J (2021) Propofol suppresses gastric cancer progression by regulating circPDSS1/miR-1324/SOX4 Axis. Cancer Manag Res 13:6031–6043. https://doi.org/10.2147/CMAR.S312989
304. Cao Y, Fan L, Li L et al (2022) Propofol suppresses cell proliferation in gastric cancer cells through NRF2-mediated polyol pathway. Clin Exp Pharmacol Physiol 49(2):264–274. https://doi.org/10.1111/1440-1681.13595
305. Liao X, Che X, Zhao W et al (2010) The beta-adrenoceptor antagonist, propranolol, induces human gastric cancer cell apoptosis and cell cycle arrest via inhibiting nuclear factor kappaB signaling. Oncol Rep 24(6):1669–1676. https://doi.org/10.3892/or_00001032
306. Liao X, Che X, Zhao W et al (2010) Effects of propranolol in combination with radiation on apoptosis and survival of gastric cancer cells in vitro. Radiat Oncol 5:98. https://doi.org/10.1186/1748-717X-5-98

307. Liao X, Chaudhary P, Qiu G et al (2018) The role of propranolol as a radiosensitizer in gastric cancer treatment. Drug Des Devel Ther 12:639–645. https://doi.org/10.2147/DDDT.S160865
308. Hu Q, Liao P, Li W et al (2021) Clinical use of propranolol reduces biomarkers of proliferation in gastric cancer. Front Oncol 11:628613. https://doi.org/10.3389/fonc.2021.628613
309. Koh M, Takahashi T, Kurokawa Y et al (2021) Propranolol suppresses gastric cancer cell growth by regulating proliferation and apoptosis. Gastric Cancer 24(5):1037–1049. https://doi.org/10.1007/s10120-021-01184-7
310. Gu M, Zhang Y, Zhou X et al (2014) Rabeprazole exhibits antiproliferative effects on human gastric cancer cell lines. Oncol Lett 8(4):1739–1744. https://doi.org/10.3892/ol.2014.2354
311. Primrose JN, Miller GV, Preston SR et al (1998) A prospective randomised controlled study of the use of ranitidine in patients with gastric cancer. Yorkshire GI Tumour Group. Gut 42(1): 17–19. https://doi.org/10.1136/gut.42.1.17
312. Chen VC, Hsu TC, Lin CF et al (2022) Association of risperidone with gastric cancer: triangulation method from cell study, animal study, and cohort study. Front Pharmacol 13: 846455. https://doi.org/10.3389/fphar.2022.846455
313. Leung WK, Bai AH, Chan VY et al (2004) Effect of peroxisome proliferator activated receptor gamma ligands on growth and gene expression profiles of gastric cancer cells. Gut 53(3): 331–338. https://doi.org/10.1136/gut.2003.021105
314. Chen BL, Yu J, Zeng ZR et al (2008) Rosiglitazone suppresses gastric carcinogenesis by up-regulating HCaRG expression. Oncol Rep 20(5):1093–1097. https://doi.org/10.3892/or_00000114
315. Zhang L, Hu JF, Li GQ et al (2012) Rosiglitazone reverses mitomycin C resistance in human gastric cancer cells. Am J Med Sci 343(5):382–387. https://doi.org/10.1097/MAJ.0b013e31822f3c63
316. Chen FZ, Mo XM, Wang QP et al (2013) Effects of rosiglitazone on the growth and lymphangiogenesis of human gastric cancer transplanted in nude mice. Oncol Rep 30(6): 2705–2712. https://doi.org/10.3892/or.2013.2704
317. Chen QY, Huang XB, Zhao YJ et al (2022) The peroxisome proliferator-activated receptor agonist rosiglitazone specifically represses tumour metastatic potential in chromatin inaccessibility-mediated FABP4-deficient gastric cancer. Theranostics 12(4):1904–1920. https://doi.org/10.7150/thno.66814
318. Manu KA, Shanmugam MK, Li F et al (2014) Simvastatin sensitizes human gastric cancer xenograft in nude mice to capecitabine by suppressing nuclear factor-kappa B-regulated gene products. J Mol Med 92(3):267–276. https://doi.org/10.1007/s00109-013-1095-0
319. Liu Q, Xia H, Zhou S et al (2020) Simvastatin Inhibits the malignant behaviors of gastric cancer cells by simultaneously suppressing YAP and beta-catenin signaling. Onco Targets Ther 13:2057–2066. https://doi.org/10.2147/OTT.S237693
320. Ortiz N, Diaz C (2020) Mevalonate pathway as a novel target for the treatment of metastatic gastric cancer. Oncol Lett 20(6):320. https://doi.org/10.3892/ol.2020.12183
321. Ortiz N, Delgado-Carazo JC, Diaz C (2021) Importance of mevalonate pathway lipids on the growth and survival of primary and metastatic gastric carcinoma cells. Clin Exp Gastroenterol 14:217–228. https://doi.org/10.2147/CEG.S310235
322. Xia Y, Jin Y, Cui D et al (2022) Antitumor effect of simvastatin in combination with DNA methyltransferase inhibitor on gastric cancer via GSDME-mediated pyroptosis. Front Pharmacol 13:860546. https://doi.org/10.3389/fphar.2022.860546
323. Kim ST, Kang JH, Lee J et al (2014) Simvastatin plus capecitabine-cisplatin versus placebo plus capecitabine-cisplatin in patients with previously untreated advanced gastric cancer: a double-blind randomised phase 3 study. Eur J Cancer 50(16):2822–2830. https://doi.org/10.1016/j.ejca.2014.08.005
324. Lang SA, Gaumann A, Koehl GE et al (2007) Mammalian target of rapamycin is activated in human gastric cancer and serves as a target for therapy in an experimental model. Int J Cancer 120(8):1803–1810. https://doi.org/10.1002/ijc.22442

325. Kamata S, Kishimoto T, Kobayashi S et al (2007) Possible involvement of persistent activity of the mammalian target of rapamycin pathway in the cisplatin resistance of AFP-producing gastric cancer cells. Cancer Biol Ther 6(7):1036–1043. https://doi.org/10.4161/cbt.6.7.4253
326. Shigematsu H, Yoshida K, Sanada Y et al (2010) Rapamycin enhances chemotherapy-induced cytotoxicity by inhibiting the expressions of TS and ERK in gastric cancer cells. Int J Cancer 126(11):2716–2725. https://doi.org/10.1002/ijc.24990
327. Kubota E, Kataoka H, Tanaka M et al (2011) ERas enhances resistance to CPT-11 in gastric cancer. Anticancer Res 31(10):3353–3360
328. Yao C, Liu J, Shao L (2011) Rapamycin inhibits the proliferation and apoptosis of gastric cancer cells by down regulating the expression of survivin. Hepato-Gastroenterology 58(107-108):1075–1080
329. Chen G, Chen SM, Wang X et al (2012) Inhibition of chemokine (CXC motif) ligand 12/chemokine (CXC motif) receptor 4 axis (CXCL12/CXCR4)-mediated cell migration by targeting mammalian target of rapamycin (mTOR) pathway in human gastric carcinoma cells. J Biol Chem 287(15):12132–12141. https://doi.org/10.1074/jbc.M111.302299
330. Chen W, Zou P, Zhao Z et al (2016) Synergistic antitumor activity of rapamycin and EF24 via increasing ROS for the treatment of gastric cancer. Redox Biol 10:78–89. https://doi.org/10.1016/j.redox.2016.09.006
331. Zhu CH, Peng SQ, Cui LL et al (2022) Synergistic effects of Rapamycin and Fluorouracil to treat a gastric tumor in a PTEN conditional deletion mouse model. Gastric Cancer 25(1):96–106. https://doi.org/10.1007/s10120-021-01229-x
332. Wang Q, Lu P, Wang T et al (2020) Sitagliptin affects gastric cancer cells proliferation by suppressing Melanoma-associated antigen-A3 expression through Yes-associated protein inactivation. Cancer Med 9(11):3816–3828. https://doi.org/10.1002/cam4.3024
333. Wang SF, Chen MS, Chou YC et al (2016) Mitochondrial dysfunction enhances cisplatin resistance in human gastric cancer cells via the ROS-activated GCN2-eIF2alpha-ATF4-xCT pathway. Oncotarget 7(45):74132–74151. https://doi.org/10.18632/oncotarget.12356
334. Miyoshi S, Tsugawa H, Matsuzaki J et al (2018) Inhibiting xCT improves 5-fluorouracil resistance of gastric cancer induced by CD44 variant 9 expression. Anticancer Res 38(11):6163–6170. https://doi.org/10.21873/anticanres.12969
335. Takizawa K, Muramatsu K, Maruyama K et al (2020) Metabolic profiling of human gastric cancer cells treated with salazosulfapyridine. Technol Cancer Res Treat 19:1533033820928621. https://doi.org/10.1177/1533033820928621
336. Zhuang J, Liu X, Yang Y et al (2021) Sulfasalazine, a potent suppressor of gastric cancer proliferation and metastasis by inhibition of xCT: conventional drug in new use. J Cell Mol Med 25(12):5372–5380. https://doi.org/10.1111/jcmm.16548
337. Shitara K, Doi T, Nagano O et al (2017) Dose-escalation study for the targeting of CD44v(+) cancer stem cells by sulfasalazine in patients with advanced gastric cancer (EPOC1205). Gastric Cancer 20(2):341–349. https://doi.org/10.1007/s10120-016-0610-8
338. Shitara K, Doi T, Nagano O et al (2017) Phase 1 study of sulfasalazine and cisplatin for patients with CD44v-positive gastric cancer refractory to cisplatin (EPOC1407). Gastric Cancer 20(6):1004–1009. https://doi.org/10.1007/s10120-017-0720-y
339. Mu C, Peng RK, Guo CL et al (2021) Discovery of sertraline and its derivatives able to combat drug-resistant gastric cancer cell via inducing apoptosis. Bioorg Med Chem Lett 41:127997. https://doi.org/10.1016/j.bmcl.2021.127997
340. Wu YL, Sun B, Zhang XJ et al (2001) Growth inhibition and apoptosis induction of Sulindac on Human gastric cancer cells. World J Gastroenterol 7(6):796–800. https://doi.org/10.3748/wjg.v7.i6.796
341. Ma L, Xie YL, Yu Y et al (2005) Apoptosis of human gastric cancer SGC-7901 cells induced by mitomycin combined with sulindac. World J Gastroenterol 11(12):1829–1832. https://doi.org/10.3748/wjg.v11.i12.1829

342. Sukumaran S, Patel HJ, Patel BM (2016) Evaluation of role of telmisartan in combination with 5-fluorouracil in gastric cancer cachexia. Life Sci 154:15–23. https://doi.org/10.1016/j.lfs.2016.04.029
343. Fujita N, Fujita K, Iwama H et al (2020) Antihypertensive drug telmisartan suppresses the proliferation of gastric cancer cells in vitro and in vivo. Oncol Rep 44(1):339–348. https://doi.org/10.3892/or.2020.7607
344. Tsujiya Y, Yamamori M, Hasegawa AI et al (2021) Telmisartan exerts cytotoxicity in scirrhous gastric cancer cells by inducing G0/G1 cell cycle arrest. Anticancer Res 41(11):5461–5468. https://doi.org/10.21873/anticanres.15358
345. Mu J, Xu H, Yang Y et al (2014) Thioridazine, an antipsychotic drug, elicits potent antitumor effects in gastric cancer. Oncol Rep 31(5):2107–2114. https://doi.org/10.3892/or.2014.3068
346. Yashiro M, Chung YS, Sowa M (1997) Tranilast (N-(3,4-dimethoxycinnamoyl) anthranilic acid) down-regulates the growth of scirrhous gastric cancer. Anticancer Res 17(2A):895–900
347. Murahashi K, Yashiro M, Inoue T et al (1998) Tranilast and cisplatin as an experimental combination therapy for scirrhous gastric cancer. Int J Oncol 13(6):1235–1240. https://doi.org/10.3892/ijo.13.6.1235
348. Yashiro M, Murahashi K, Matsuoka T et al (2003) Tranilast (N-3,4-dimethoxycinamoyl anthranilic acid): a novel inhibitor of invasion-stimulating interaction between gastric cancer cells and orthotopic fibroblasts. Anticancer Res 23(5A):3899–3904
349. Nakajima K, Okita Y, Matsuda S (2004) Sensitivity of scirrhous gastric cancer to 5-fluorouracil and the role of cancer cell-stromal fibroblast interaction. Oncol Rep 12(1):85–90
350. Laurino S, Mazzone P, Ruggieri V et al (2021) Cationic channel TRPV2 overexpression promotes resistance to cisplatin-induced apoptosis in gastric cancer cells. Front Pharmacol 12:746628. https://doi.org/10.3389/fphar.2021.746628
351. Wang J, Chen X, Su L et al (2016) Suppressive effects on cell proliferation and motility in gastric cancer SGC-7901 cells by introducing ulinastatin in vitro. Anti-Cancer Drugs 27(7):651–659. https://doi.org/10.1097/CAD.0000000000000378
352. Lim SC, Duong HQ, Choi JE et al (2011) Lipid raft-dependent death receptor 5 (DR5) expression and activation are critical for ursodeoxycholic acid-induced apoptosis in gastric cancer cells. Carcinogenesis 32(5):723–731. https://doi.org/10.1093/carcin/bgr038
353. Lim SC, Han SI (2015) Ursodeoxycholic acid effectively kills drug-resistant gastric cancer cells through induction of autophagic death. Oncol Rep 34(3):1261–1268. https://doi.org/10.3892/or.2015.4076
354. Yagi Y, Fushida S, Harada S et al (2010) Effects of valproic acid on the cell cycle and apoptosis through acetylation of histone and tubulin in a scirrhous gastric cancer cell line. J Exp Clin Cancer Res 29:149. https://doi.org/10.1186/1756-9966-29-149
355. Sun J, Piao J, Li N et al (2020) Valproic acid targets HDAC1/2 and HDAC1/PTEN/Akt signalling to inhibit cell proliferation via the induction of autophagy in gastric cancer. FEBS J 287(10):2118–2133. https://doi.org/10.1111/febs.15122
356. Amnekar RV, Khan SA, Rashid M et al (2020) Histone deacetylase inhibitor pre-treatment enhances the efficacy of DNA-interacting chemotherapeutic drugs in gastric cancer. World J Gastroenterol 26(6):598–613. https://doi.org/10.3748/wjg.v26.i6.598
357. Jahani M, Khanahmad H, Nikpour P (2021) Evaluation of the effects of valproic acid treatment on cell survival and epithelial-mesenchymal transition-related features of human gastric cancer cells. J Gastrointest Cancer 52(2):676–681. https://doi.org/10.1007/s12029-019-00332-8
358. Fushida S, Kinoshita J, Kaji M et al (2016) Paclitaxel plus valproic acid versus paclitaxel alone as second- or third-line therapy for advanced gastric cancer: a randomized Phase II trial. Drug Des Devel Ther 10:2353–2358. https://doi.org/10.2147/DDDT.S110425
359. Shchepotin IB, Shabahang M, Nauta RJ et al (1994) Antitumour activity of 5-fluorouracil, verapamil and hyperthermia against human gastric adenocarcinoma cell (AGS) in vitro. Surg Oncol 3(5):287–294. https://doi.org/10.1016/0960-7404(94)90031-0

360. Shchepotin IB, Buras RR, Nauta RJ et al (1994) Effect of mitomycin C, verapamil, and hyperthermia on human gastric adenocarcinoma. Cancer Chemother Pharmacol 34(3): 257–260. https://doi.org/10.1007/BF00685086
361. Nozue M, Nishida M, Todoroki T et al (1995) Selection of three out of 24 anti-cancer agents in poorly-differentiated gastric cancer cell lines, evaluated by the AUC/delta IC50 ratio. Anti-Cancer Drugs 6(2):291–302. https://doi.org/10.1097/00001813-199504000-00014
362. Wang X, Li Y, Fan GF et al (2020) Effect of verapamil in the reversal of doxorubicin chemotherapy resistance in advanced gastric cancer. Eur Rev Med Pharmacol Sci 24(14): 7753–7763. https://doi.org/10.26355/eurrev_202007_22278
363. Ning Z, Chen D, Liu A et al (2014) Efficacy of chemotherapy combined with targeted arterial infusion of verapamil in patients with advanced gastric cancer. Cell Biochem Biophys 68(1): 195–200. https://doi.org/10.1007/s12013-013-9689-2
364. Kang MH, Jeong GS, Smoot DT et al (2017) Verteporfin inhibits gastric cancer cell growth by suppressing adhesion molecule FAT1. Oncotarget 8(58):98887–98897. https://doi.org/10.18632/oncotarget.21946
365. Xiong J, Wang S, Chen T et al (2019) Verteporfin blocks Clusterin which is required for survival of gastric cancer stem cell by modulating HSP90 function. Int J Biol Sci 15(2): 312–324. https://doi.org/10.7150/ijbs.29135
366. Giraud J, Molina-Castro S, Seeneevassen L et al (2020) Verteporfin targeting YAP1/TAZ-TEAD transcriptional activity inhibits the tumorigenic properties of gastric cancer stem cells. Int J Cancer 146(8):2255–2267. https://doi.org/10.1002/ijc.32667
367. Hasegawa T, Sugihara T, Hoshino Y et al (2021) Photosensitizer verteporfin inhibits the growth of YAP- and TAZ-dominant gastric cancer cells by suppressing the anti-apoptotic protein Survivin in a light-independent manner. Oncol Lett 22(4):703. https://doi.org/10.3892/ol.2021.12964
368. Zheng QS, Sun XL, Wang CH (2002) Redifferentiation of human gastric cancer cells induced by ascorbic acid and sodium selenite. Biomed Environ Sci 15(3):223–232
369. Oliveira CP, Kassab P, Lopasso FP et al (2003) Protective effect of ascorbic acid in experimental gastric cancer: reduction of oxidative stress. World J Gastroenterol 9(3):446–448. https://doi.org/10.3748/wjg.v9.i3.446
370. Sun YX, Zheng QS, Li G et al (2006) Mechanism of ascorbic acid-induced reversion against malignant phenotype in human gastric cancer cells. Biomed Environ Sci 19(5):385–391
371. Ha YM, Park MK, Kim HJ et al (2009) High concentrations of ascorbic acid induces apoptosis of human gastric cancer cell by p38-MAP kinase-dependent up-regulation of transferrin receptor. Cancer Lett 277(1):48–54. https://doi.org/10.1016/j.canlet.2008.11.020
372. Nagappan A, Park KI, Park HS et al (2012) Vitamin C induces apoptosis in AGS cells by down-regulation of 14-3-3sigma via a mitochondrial dependent pathway. Food Chem 135(3): 1920–1928. https://doi.org/10.1016/j.foodchem.2012.06.050
373. Lu YX, Wu QN, Chen DL et al (2018) Pharmacological ascorbate suppresses growth of gastric cancer cells with GLUT1 overexpression and enhances the efficacy of oxaliplatin through redox modulation. Theranostics 8(5):1312–1326. https://doi.org/10.7150/thno.21745
374. O'Leary BR, Houwen FK, Johnson CL et al (2018) Pharmacological ascorbate as an adjuvant for enhancing radiation-chemotherapy responses in gastric adenocarcinoma. Radiat Res 189(5):456–465. https://doi.org/10.1667/RR14978.1
375. Ghavami G, Sardari S (2020) Synergistic effect of vitamin C with cisplatin for inhibiting proliferation of gastric cancer cells. Iran Biomed J 24(2):119–127
376. Chen D, Wei X, Yang K et al (2022) Piperlongumine combined with vitamin C as a new adjuvant therapy against gastric cancer regulates the ROS-STAT3 pathway. J Int Med Res 50(4):3000605221093308. https://doi.org/10.1177/03000605221093308
377. Wang GQ, Dawsey SM, Li JY et al (1994) Effects of vitamin/mineral supplementation on the prevalence of histological dysplasia and early cancer of the esophagus and stomach: results from the General Population Trial in Linxian, China. Cancer Epidemiol Biomarkers Prev 3(2): 161–166

378. Tsubono Y, Okubo S, Hayashi M et al (1997) A randomized controlled trial for chemoprevention of gastric cancer in high-risk Japanese population; study design, feasibility and protocol modification. Jpn J Cancer Res 88(4):344–349. https://doi.org/10.1111/j.1349-7006.1997.tb00387.x
379. Wang F, He MM, Wang ZX et al (2019) Phase I study of high-dose ascorbic acid with mFOLFOX6 or FOLFIRI in patients with metastatic colorectal cancer or gastric cancer. BMC Cancer 19(1):460. https://doi.org/10.1186/s12885-019-5696-z
380. Baek S, Lee YS, Shim HE et al (2011) Vitamin D3 regulates cell viability in gastric cancer and cholangiocarcinoma. Anat Cell Biol 44(3):204–209. https://doi.org/10.5115/acb.2011.44.3.204
381. Park MR, Lee JH, Park MS et al (2012) Suppressive effect of 19-nor-1alpha-25-dihydroxyvitamin D2 on gastric cancer cells and peritoneal metastasis model. J Korean Med Sci 27(9):1037–1043. https://doi.org/10.3346/jkms.2012.27.9.1037
382. Bao A, Li Y, Tong Y et al (2013) Tumor-suppressive effects of 1, 25-dihydroxyvitamin D3 in gastric cancer cells. Hepato-Gastroenterology 60(124):943–948. https://doi.org/10.5754/hge121003
383. Bao A, Li Y, Tong Y et al (2014) 1,25-Dihydroxyvitamin D(3) and cisplatin synergistically induce apoptosis and cell cycle arrest in gastric cancer cells. Int J Mol Med 33(5):1177–1184. https://doi.org/10.3892/ijmm.2014.1664
384. Chang S, Gao L, Yang Y et al (2015) miR-145 mediates the antiproliferative and gene regulatory effects of vitamin D3 by directly targeting E2F3 in gastric cancer cells. Oncotarget 6(10):7675–7685. https://doi.org/10.18632/oncotarget.3048
385. Li M, Li L, Zhang L et al (2017) 1,25-Dihydroxyvitamin D3 suppresses gastric cancer cell growth through VDR- and mutant p53-mediated induction of p21. Life Sci 179:88–97. https://doi.org/10.1016/j.lfs.2017.04.021
386. Wu J, Zhao Q, Zhao Y et al (2021) Dicer increases the indication for trastuzumab treatment in gastric cancer patients via overexpression of human epidermal growth factor receptor 2. Sci Rep 11(1):6993. https://doi.org/10.1038/s41598-021-86485-8
387. Urashima M, Ohdaira H, Akutsu T et al (2019) Effect of vitamin D supplementation on relapse-free survival among patients with digestive tract cancers: the AMATERASU randomized clinical trial. JAMA 321(14):1361–1369. https://doi.org/10.1001/jama.2019.2210
388. Lee MH, Cho Y, Kim DH et al (2016) Menadione induces G2/M arrest in gastric cancer cells by down-regulation of CDC25C and proteasome mediated degradation of CDK1 and cyclin B1. Am J Transl Res 8(12):5246–5255
389. Bona AB, Calcagno DQ, Ribeiro HF et al (2020) Menadione reduces CDC25B expression and promotes tumor shrinkage in gastric cancer. Ther Adv Gastroenterol 13:1756284819895435. https://doi.org/10.1177/1756284819895435
390. Yasuda C, Kato M, Kuroda D et al (1997) Experimental studies on potentiation of the antitumor activity of 5-fluorouracil with 3′-azido-3′-deoxythymidine for the gastric cancer cell line MKN28 in vivo. Jpn J Cancer Res 88(1):97–102. https://doi.org/10.1111/j.1349-7006.1997.tb00307.x
391. Sun YQ, Guo TK, Xi YM et al (2007) Effects of AZT and RNA-protein complex (FA-2-b-beta) extracted from Liang Jin mushroom on apoptosis of gastric cancer cells. World J Gastroenterol 13(31):4185–4191. https://doi.org/10.3748/wjg.v13.i31.4185
392. Kim BJ, Kim SY, Lee S et al (2012) The role of transient receptor potential channel blockers in human gastric cancer cell viability. Can J Physiol Pharmacol 90(2):175–186. https://doi.org/10.1139/y11-114
393. Tang J, Zhang C, Lin J et al (2021) ALOX5-5-HETE promotes gastric cancer growth and alleviates chemotherapy toxicity via MEK/ERK activation. Cancer Med 10(15):5246–5255. https://doi.org/10.1002/cam4.4066

The Chemoprevention of Hereditary Diffuse Gastric Cancer

21

Lyvianne Decourtye-Espiard and Parry Guilford

Abstract

Mutations in the *CDH1* gene are the genetic hallmark of hereditary diffuse gastric cancer (HDGC), a cancer syndrome characterised by high rates of both diffuse gastric cancer and lobular breast cancer. Clinical guidelines recommend that *CDH1* mutation carriers undergo a prophylactic gastrectomy, or, alternatively, endoscopic surveillance in a centre with established HDGC experience. However, both management options have limitations: the short- and long-term morbidity associated with a gastrectomy, and the risk of developing an interval cancer during surveillance. These limitations compel the identification of chemoprevention drugs which can be used to either mitigate the need for surgery or complement surveillance. In this chapter, we will discuss progress in identifying druggable vulnerabilities in E-cadherin-null cells, strategies to increase the tolerability of HDGC chemoprevention, and pathways for drug validation and clinical testing.

21.1 Introduction

Hereditary diffuse gastric cancer (HDGC) is a cancer syndrome characterised by a high incidence of both diffuse-type gastric cancer (DGC) and lobular breast cancer (LBC). HDGC is predominantly caused by germline mutations in the *CDH1* gene encoding the cell–cell adhesion protein E-cadherin [1, 2]. *CDH1* mutation carriers' lifetime risk of DGC varies considerably between families, suggesting the existence of risk modifier genes and environmental factors that modulate disease progression.

L. Decourtye-Espiard · P. Guilford (✉)
Cancer Genetics Laboratory, Department of Biochemistry, Centre for Translational Cancer Research (Te Aho Matatū), University of Otago, Dunedin, New Zealand
e-mail: parry.guilford@otago.ac.nz

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_21

Risk estimates in selected HDGC families are as high as 67% and 83% for men and women, respectively [3]. However, it is likely that the lifetime risk of DGC is lower for most *CDH1* mutation carriers, with estimates ranging from 25–33% for women and 37–42% for men [4, 5]. Estimates of LBC penetrance in female germline *CDH1* mutation carriers are less variable, ranging from 39 to 55% [3–6].

HDGC clinical guidelines recommend that carriers of pathogenic *CDH1* mutations with a personal or family history of DGC undergo a prophylactic gastrectomy [2]. For those declining surgery or wishing to delay the procedure, annual endoscopic surveillance in an expert HDGC centre is recommended. However, both management options present difficulties: gastrectomies frequently result in immediate complications such as anastomotic strictures and bile reflux, but also longer-term sequelae that include nutritional, hormonal, neurocognitive, pharmacokinetic, and psychological effects; surveillance runs the risk of failing to identify cancers that may have begun to spread under an intact mucosal surface, a risk that is amplified by the accumulated mucosal scarring caused by the large number of targeted and random biopsies that are taken during gastric surveillance [2, 7]. Female *CDH1* mutation carriers are also advised to undergo breast surveillance from 30 years of age, with consideration given to risk-reducing mastectomy. Again, these options have risks and consequences—the detection of LBC is more difficult than the ductal subtype of breast cancer, and women who have undergone a mastectomy can suffer from long-term lymphedema and psychological issues [8, 9].

Although the existing management methods for HDGC have saved hundreds of lives worldwide, the morbidity and long-term sequelae associated with prophylactic surgery or, alternatively, the risk presented by reliance on surveillance alone, compel the development of chemoprevention strategies that can mitigate the need for surgery or complement surveillance. Chemoprevention provides a means to manage cancer risk not only in HDGC families with obvious high risk, but also in *CDH1* mutation carriers whose family history is suggestive of reduced mutation penetrance (due to modifier genes or altered environment). Such lower risk families are perennial problems for cancer genetic counselling, leading to the dichotomy of potentially over-treating or under-treating an individual. In HDGC, this clinical dilemma is typified by pathogenic *CDH1* mutation carriers who have a moderate family history of LBC but little or no DGC. Chemoprevention might also be a valuable tool for some carriers of *CDH1* variants of unknown significance. These variants carriers, who outnumber those with pathogenic or likely pathogenic *CDH1* mutations 4:1 (ncbi.nlm.nih.gov/clinvar/), are not offered a prophylactic gastrectomy and face an uncertain, and unsettling, risk of disease progression.

21.2 HDGC Pathology

The pathological hallmark of HDGC is multifocal, stage T1a signet ring cell carcinomas (SRCC) in the gastric mucosa, with up to several hundred foci/stomach (Fig. 21.1) [10]. These SRCC foci, which are present from an early age, have an average diameter of <1 mm and are localised to the *lamina propria* below an intact

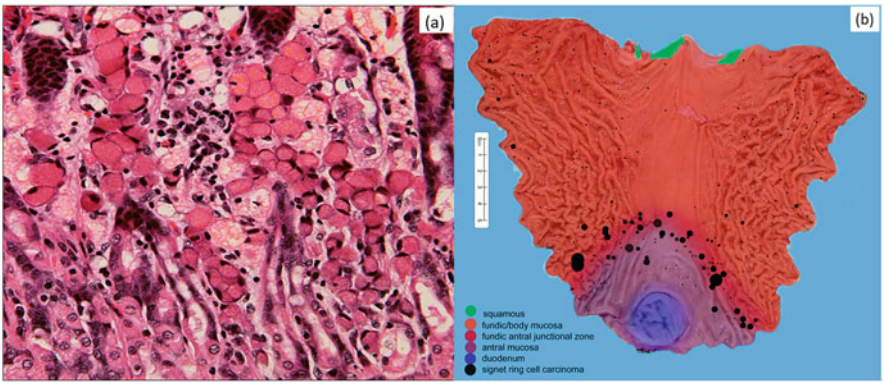


Fig. 21.1 (**a**) Haematoxylin- and eosin-stained gastric mucosa showing signet ring cells in the lamina propria. (**b**) Anatomical map showing the stomach mucosal zones and location and size of SRCC foci in a prophylactic gastrectomy specimen. Originally published in [11]

mucosa [11]. The foci are formed following somatic inactivation of the second *CDH1* allele, frequently by promoter hypermethylation [12, 13]. The enrichment of foci in the gastric transition zone in a subset of mutation carriers illustrates the importance of epigenetic regulation to the initiation of foci throughout the stomach (Fig. 21.1b) [11].

Elimination of E-cadherin-mediated cell-to-cell adhesion leads to a loss of cell polarity and subsequent misalignment of the mitotic spindle during cell division [14–18]. It is hypothesised that this misalignment leads to the division of a subset of stem or progenitor cells out of the epithelial plane and into the *lamina propria* where they develop into SRCCs [14, 19]. SRCCs have a lower mitotic index than surrounding tissue and display Ki67 negativity [20]. These indolent foci can remain at stage T1a for many years [21, 22] and a proportion may even be transient. However, following additional mutations and/or an epithelial-mesenchymal transition, early SRCCs will invade extensively through the stomach's underlying muscle layers before dissemination into the peritoneal cavity or distant metastatic sites [12, 20]. Although the additional mutations that drive the progression of stage T1a SRCCs have not yet been described, the high frequency of *TP53* mutations in sporadic DGC and the importance of *Tp53* mutations to the establishment of mouse gastric cancer models suggest that mutations in this gene will be common drivers of progression [23].

The natural history of breast cancer in HDGC is less well described; however, it is clear that pathogenic germline *CDH1* mutations also lead to multifocal disease comprising foci of atypical lobular hyperplasia, lobular carcinoma in situ (LCIS), and LBC [24–26].

21.3 Is HDGC Amenable to Chemoprevention?

In general, the concept of cancer chemoprevention is daunting. Chemoprevention drugs must not only kill tumour cells or prevent their initiation, but must also be sufficiently well tolerated to be administered over extended periods, perhaps many years. For HDGC, there are two possible approaches to chemoprevention. One involves the use of drugs which block cancer formation by preventing the epigenetic inactivation of the 2nd *CDH1* allele. Although the prevention of cancer initiation has a fundamental appeal, this approach would be expected to require the near-constant maintenance of an active drug concentration, demanding exceptional drug tolerability to ensure long-term compliance. Another anticipated drawback would be the inhibitory effect that 'locked' *CDH1* expression would have on normal cell migration during tissue repair [27]. Moreover, it seems improbable that the same drug that prevents epigenetic downregulation would be able to prevent 2nd *CDH1* allele loss through the distinct mechanisms of mutation or deletion, should they occur [28].

The second possible approach to HDGC chemoprevention involves killing *CDH1*-null breast and gastric epithelial cells before they have acquired additional growth characteristics and, in the case of stomach tissue, before the foci of *CDH1*-null signet ring cells have progressed beyond stage T1a. This chemoprevention approach requires the near obligatory use of cytotoxic drugs. However, such drugs would need to be administered relatively infrequently, perhaps yearly. Mutation carriers are therefore more likely to find this approach acceptable in the long term. Uncertainties with this approach include the efficacy of candidate drugs on different epithelial cell lineages and our incomplete understanding of the genetic drivers of HDGC progression.

Another major challenge facing HDGC chemoprevention, unlike other cancer syndromes such as familial adenomatous polyposis, is the difficulty in quantifying successful chemoprevention. This difficulty is due to our current inability to detect the majority of the early-stage target lesions in vivo, in either the stomach or breast. Another challenge, but this time common to familial adenomatous polyposis chemoprevention, is presented by the question 'what relative reduction in the number of cancer foci would be sufficient to change a mutation carrier's perception of risk?' Or alternatively, 'what level of reduced risk would be sufficient to change clinical recommendations for a prophylactic gastrectomy?' Although the answer to this question is subjective, an order of magnitude reduction in risk is likely to be a minimum target.

Balanced against these challenges are several features of HDGC which argue in favour of the successful application of chemoprevention. Firstly, the size, structure, and genetics of the gastric stage T1a SRCCs are all amenable to drug treatment. The great majority of these foci are <1 mm in diameter [11] and are therefore readily accessible to drug and lacking in a drug-resistant, hypoxic core. The large number of independent gastric SRCC foci in individual mutation carriers argue that mutation of *CDH1* alone is sufficient for tumour initiation, although additional epigenetic events to other genes may contribute. This mutational homogeneity implies greater predictability of drug response and also enables better selection of representative cell line,

organoid, and mouse models. The absence of mutational heterogeneity and the small total tumour burden in most *CDH1* mutation carriers also reduces the likelihood of pre-existing drug resistance mutations. Finally, and perhaps most importantly, the relative indolence of the stage T1a SRCC translates to low clinical urgency, suggesting that large pharmacological indices will not be required; i.e. drugs, and drug doses, that slowly reduce tumour fitness relative to normal tissue may be sufficient to eradicate disease.

21.4 Targeting *CDH1*-Null Epithelial Cells

A cytotoxic chemoprevention strategy for HDGC relies on the identification of druggable vulnerabilities that are established following the loss of E-cadherin from the cell. Such vulnerabilities are frequently referred to as being 'synthetic lethal'. In classical genetics, synthetic lethality defines a relationship between two genes in which mutations in both genes at the same time result in cell death, but mutation of either gene alone does not. In a therapeutic setting, an antagonistic drug substitutes for mutation of one gene in a synthetic lethal pair, leading to cell death when the second gene (e.g. *CDH1*) is mutated. In oncology, the effects of a synthetic lethal drug are not necessarily as binary, as they may just cause a relative reduction in the growth or fitness of mutated cells compared to wild type.

To begin to identify the vulnerabilities in *CDH1*-mutant cells, much early work has focused on characterising isogenic MCF10A cells with and without *CDH1*. MCF10A is a spontaneous, non-malignant breast cell with a relatively stable genome and few background mutations [29, 30]. It therefore provides a reasonable model for isolating the consequences of *CDH1* inactivation from other genetic events. The MCF10A isogenic pair was generated by homozygous, frameshift deletion of 4 bp in exon 11 of *CDH1* (Sigma-Aldrich, Saint Louis). MCF10A-$CDH1^{-/-}$ cells display distinct differences in the cytoskeleton, gene expression patterns, and RNAi sensitivity, providing a canvas for potential targetable vulnerabilities.

Cytoskeletal Changes MCF10A-$CDH1^{-/-}$ cells exhibit clear changes in cytoskeletal organisation relative to wild-type cells. Filamentous actin forms thicker, more numerous stress fibres in the basal part of the $CDH1^{-/-}$ cells. In addition, the apical microtubule network lacks the radial pattern of organisation observed in wild-type cells and the microtubules are often orientated parallel to the cell cortex [31]. Notably, $CDH1^{-/-}$ cells also have significantly fewer nucleoli/cell, suggesting a decreased demand or capability for ribosome biogenesis [31, 32].

Gene Expression Changes E-cadherin loss from MCF10A cells causes a significant shift in the transcriptome, with one study reporting the differential regulation of 1388 genes [31]. These expression changes include other cadherin family members, with *CDH2* and *CDH4* downregulated by >twofold and *CDH3* and *CDH16* upregulated nearly fourfold. E-cadherin loss also upregulates multiple tight junction, desmosome, and gap junction proteins. These changes may in part compensate for

the weakened E-cadherin-mediated cell-to-cell adhesion and explain the maintenance of a cobblestone morphology in MCF10A-*CDH1*$^{-/-}$ cells at full confluency [31]. Gene expression data however also point to coordinate transcriptional changes that could weaken adhesion to the cell substrate, including the downregulation of several integrin family members (such as *ITGA1, ITGA4, ITGB1,* and *ITGB2*) and extracellular matrix (ECM) proteins, including multiple collagens, laminins, fibronectin, and vitronectin. Moreover, a direct link between E-cadherin regulation and tissue remodelling was demonstrated by the increased expression of *MMP9*, *MMP14*, multiple kallikrein proteases and decreased expression of *TIMP2* and *TIMP3*. Together, these transcription changes highlight that E-cadherin loss impacts not only on cell-cell adhesion but also cell-substrate adhesion, and suggest that these interactions may harbour druggable vulnerabilities.

RNAi Sensitivity To systematically catalogue vulnerabilities created by E-cadherin loss, Telford et al. performed a genome-wide siRNA knockdown of more than 18,000 genes using isogenic *CDH1* MCF10A cells [33]. Synthetic lethality was determined by measuring the relative viability of *CDH1*-null and *CDH1*-expressing cells following knockdown. Remarkably, the loss of E-cadherin affected entire classes of transmembrane proteins, not just proteins with a predictable association with E-cadherin [33, 34]. For example, knockdown of 245 non-sensory GPCRs showed a striking bimodal effect on cell viability, with most GPCR siRNAs either increasing or decreasing the relative viability of the *CDH1*-null cells, and remarkably few having no impact. The GPCR siRNAs were split almost equally between those that reduced the viability of *CDH1*-null cells relative to wild-type cells ('synthetic lethal') and those that were more inhibitory to the wild-type cells ('reverse synthetic lethal'). The reverse synthetic lethal group are hypothesised to correspond to proteins which have functional homologues that are upregulated in *CDH1*-null cells and compensate for the siRNA-mediated downregulation of the target protein. On a similar scale to the GPCRs, the individual knockdown of 161 voltage-gated ion channel genes resulted in reverse synthetic lethal effects for most genes. The other major class of cell surface receptors, receptor kinases ($n = 75$) were, on average, synthetic lethal (Guilford, unpublished data). Together, this knockdown data on hundreds of transmembrane proteins with diverse signalling and membrane transport functions suggest that the loss of E-cadherin impacts on the functionality of the plasma membrane in a fundamental way [34].

In addition to transmembrane protein classes, processes tightly associated with vesicle trafficking also showed differential RNAi sensitivity in *CDH1*-null cells, with ubiquitination, proteosome function, endocytosis, and membrane curvature genes all showing either synthetic lethal or reverse synthetic lethal class effects. The existence of a deficit in endocytosis in E-cadherin-null cells was confirmed experimentally by a reduced capacity for cholera toxin uptake [34, 35].

Three signalling pathways have been strongly associated with E-cadherin-mediated cell-to-cell adhesion: PI3K/AKT, WNT, and HIPPO [36–40]. The genes comprising the PI3K/AKT pathway were significantly synthetic lethal in the MCF10A isogenic cell line pair, providing early evidence that this pathway is a

promising target for chemoprevention. In contrast, the WNT and HIPPO pathways showed no evidence of synthetic lethality in this model.

Overall, genome-wide RNAi sensitivity data demonstrate that the consequences of E-cadherin loss are not limited to changes in cell adhesion and cytoskeletal organisation, but also include fundamental changes to plasma membrane organisation and the efficiency of vesicle trafficking.

21.5 A Model of E-Cadherin-Null Cell Vulnerability

The above observations suggest an overarching model for *CDH1*-associated synthetic lethality (Fig. 21.2). In this model, the loss of interactions between E-cadherin and the actin and microtubule cytoskeletal networks disrupt the spatial cues required for the normal architecture and functionality of the plasma membrane [41]. This disruption is hypothesised to extend to the cholesterol and sphingolipid-enriched plasma membrane domains known as lipid rafts. Lipid rafts promote the clustering and intracellular scaffolding of classes of transmembrane proteins including cell surface receptors (such as GPCRs and receptor tyrosine kinases) and voltage-gated ion channels [42–44]. Abrogation of the interaction between E-cadherin and the cortical actin cytoskeleton may also compromise endocytosis and membrane vesicle trafficking by disrupting the forces that are required to drive plasma membrane bending and vesicle formation. Inefficient vesicle formation would disrupt vesicle trafficking to various sub-cellular compartments, including ubiquitin-directed

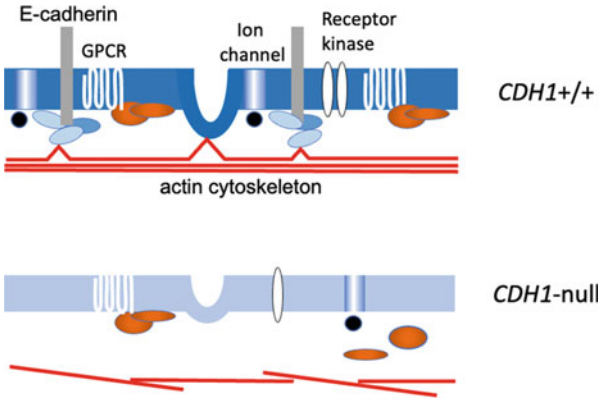


Fig. 21.2 Model of the impact of E-cadherin loss on the plasma membrane and associated protein complexes. Upon loss of E-cadherin, interactions between the plasma membrane, cortical actin filaments, and other cytoskeletal structures are disrupted. This disruption affects the ability of the membrane to efficiently self-assemble lipid rafts. As a result, the concentration of receptors and ion channels into confined areas is reduced, affecting the crosstalk between diverse membrane-associated proteins and the efficient assembly of protein scaffold complexes on the inner membrane, disrupting downstream cell signalling. Disruption of the actin cytoskeleton's interaction with the plasma membrane also diminishes the efficiency of endocytosis, impacting on vesicle trafficking throughout the cell

trafficking to the lysozyme. Reduced endocytosis efficiency might also contribute to reduced transmembrane receptor activity by inhibiting receptor recycling to and from the plasma membrane.

This model is supported by small molecule antagonists that target relevant cellular processes. For example, *CDH1*$^{-/-}$ cells are more sensitive than wild-type cells to inhibitors of actin polymerisation (cytochalasin D, latrunculin B), sphingolipid metabolism and signalling (GW-4869, PF-543), the depletion of membrane cholesterol with methyl-β-cyclodextrin and HMG-CoA reductase inhibitors (e.g. atorvastatin), and the inhibition of vesicle trafficking (e.g. bafilomycin A, chloroquine, hydroxy-chloroquine) [34, 45, 46]. The model is also supported by an RNAseq analysis of 415 advanced gastric tumours from the TCGA dataset which demonstrated that Reactome pathways predominantly involved in cell-cell adhesion, membrane trafficking, GPCR signalling, and membrane lipid composition were in a synthetic lethal relationship with E-cadherin [34].

21.6 Synthetic Lethal Drug Screens

Two high-throughput drug screens aimed at identifying HDGC chemoprevention drugs have been conducted, both using the *CDH1* isogenic MCF10A cell line pair. Beetham et al. (2019) screened the WECC library of 113,945 lead-like compounds, condensing the synthetic lethal hits down to four lead compounds that belonged to distinct pharmacophore groups [47]. Using a structure–activity relationship approach, Luzenburger et al. increased the potency and selectivity of one of these compounds (SLEC-11). At low micromolar concentrations, the derivative compound (AL-GDa62) preferentially induced apoptosis in *CDH1*-null cells and inhibited SUMOylation, suggesting this post-translational modification might be a vulnerability of *CDH1*-null cells. In a more targeted study, Telford et al. screened the Selleck Chemistry inhibitor library (326 compounds), the SYNthesis Medicinal Chemistry kinase inhibitor library (131 compounds), and the WEHI known drug library (3600 compounds) [33]. Twenty-two drugs with EC50 values 10–50% lower in the *CDH1*-null cells compared to wild-type were identified. These drugs included multiple histone deacetylase (HDAC) inhibitors, PI3K/AKT inhibitors, the receptor tyrosine kinase inhibitor crizotinib, an inhibitor of the GPCR neuropeptide receptor NPY5R (CGP71683), and the SRC family inhibitor saracatinib. Several of these classes of inhibitors have been tested more extensively (Fig. 21.3) in cell line and mouse organoid models (Fig. 21.4a) and are described in further detail below.

21.7 Candidate Chemoprevention Drugs

HDAC inhibitors: The pan-HDAC inhibitors entinostat, pracinostat, and mocetinostat are more toxic to *CDH1*-null cells relative to wild-type cells across a variety of pre-clinical models, acting through both cytostatic and pro-apoptotic mechanisms [48]. Entinostat maintains this synthetic lethal effect in *Cdh1*-null

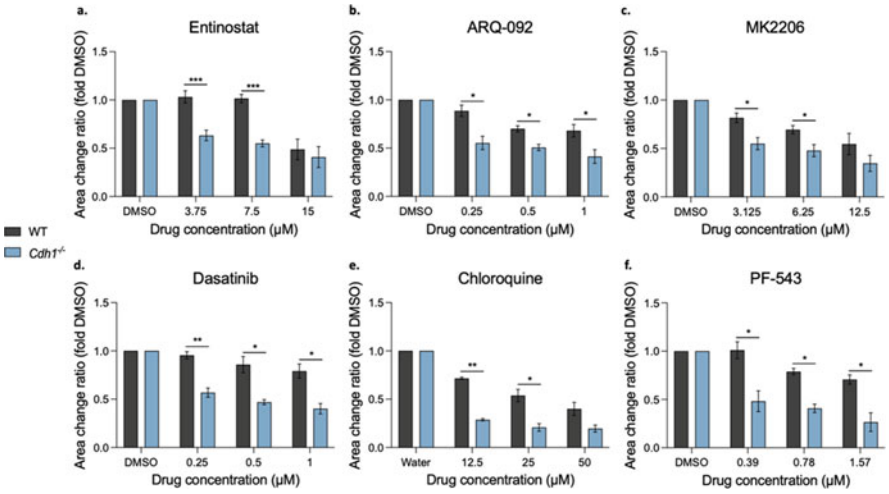


Fig. 21.3 Normalised area of wild-type (WT) and *Cdh1*$^{-/-}$ mouse gastric organoids after treatment with (**a**) entinostat, (**b**) ARQ-092, (**c**) MK2206, (**d**) dasatinib, (**e**) chloroquine, and (**f**) PF-543 Graphs are adapted from data originally published in Decourtye-Espiard et al. [48], Bougen-Zhukov et al. [49, 50] and Brew et al. [46]

mouse gastric organoids in the presence of an additional *Tp53* mutation, supporting this drug as a promising candidate for HDGC chemoprevention. Some class-specific HDAC inhibitors were also able to preferentially inhibit the growth of *CDH1*-null cells. However, these effects were not consistent across different genetic backgrounds, highlighting the greater robustness of the pan inhibitors, in particular entinostat [48].

Notably, entinostat is able to re-express epigenetically silenced *CDH1* in cancer cell lines [51, 52] and mouse gastric organoids (Fig. 21.4b) [48]. This observation suggests that it could act as an HDGC chemoprevention compound through both approaches discussed earlier, that is by (1) maintaining expression of the wild-type *CDH1* allele and thereby reducing the rate of initiation of SRCC foci, and (2) causing death of T1a SRCC foci. Phase 1 studies have shown that entinostat is generally well tolerated, although its side effects at the current therapeutic doses would probably be too severe for it to be used orally for routine *CDH1* re-expression [53, 54]. Entinostat is currently being tested in phase 2/3 trials for the treatment of multiple cancer types, both as a single agent and in combination.

SRC Family Inhibitors The SRC family of non-receptor tyrosine kinases is comprised of eleven members, including SRC, FYN, LCK, HCK, and YES [55]. In addition to saracatinib which was identified as synthetic lethal in the know drug screen [33], three other non-specific SRC family inhibitors, PP1, PP2, and SU6656, also preferentially inhibit the growth of *CDH1*-null MCF10A cells

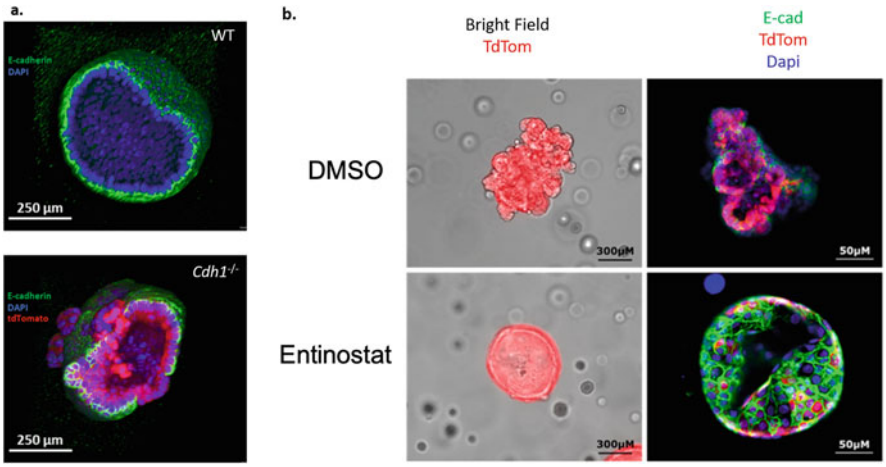


Fig. 21.4 Mouse Cd44-cre/*Cdh1*/TdTomato gastric organoid model. In this model, cre recombinase is expressed as a fusion with a mutated form of the oestrogen receptor ERT2 under the control of the *Cd44* promoter. ERT2 binds the synthetic oestrogen receptor ligand endoxifen (but not endogenous oestradiol), leading to translocation of the recombinase to the nucleus and deletion of DNA sequences that are flanked by loxP sites (fl). LoxP sites flank exons 6–10 of one, or both, *Cdh1* alleles and a reading frame-disrupting sequence engineered into the red fluorescent marker gene tdTomato. Induction with endoxifen leads to inactivation of the floxed *Cdh1* allele (s) and restoration of the tdTomato reading frame. (**a**) Confocal imaging of immunofluorescence in organoids with two floxed *Cdh1* alleles (Cd44-cre/*Cdh1*$^{fl/fl}$/Tdtomato$^{fl/fl}$). Upper panel, uninduced organoid (WT); E-cadherin (green), nuclei (DAPI blue). Lower panel, organoid after induction with endoxifen (*Cdh1*$^{-/-}$) showing E-cadherin-null cells displaced out of the epithelial plane. Originally published in Brew et al. (2021). (**b**) Bright field and immunofluorescent confocal imaging showing the re-expression of E-cadherin following entinostat treatment of endoxifen-induced organoids heterozygous for the floxed *Cdh1* allele (Cd44-cre/*Cdh1*$^{fl/WT}$/Tdtomato$^{fl/fl}$). Upper panels, vehicle only (DMSO). Lower panels, entinostat treatment. Left side, bright field imaging; right side, immunofluorescence. E-cadherin (green), nuclei (DAPI blue). Originally published in Decourtye-Espiard et al. [48]

[46]. Notably, preferential growth inhibition was not observed with bosutinib, a specific inhibitor of SRC itself, nor with a specific inhibitor of the SRC homologue LCK. These results demonstrate that the SRC family is an important target for HDGC, but functional redundancy between SRC family members may necessitate the use of pan-SRC family inhibitors for chemoprevention.

Although PP1, PP2, and SU6656 show strong *CDH1* synthetic lethality, they are toolbox drugs that have not yet been developed clinically. Fortunately, another potent inhibitor of SRC [56], dasatinib, is well-established clinically and FDA-approved for several cancer indications. Dasatinib was originally developed to inhibit the BCR-ABL fusion, the major cause of chronic myeloid and lymphoblastic leukaemias [57], but it also antagonises many other tyrosine kinases including DDR2, a collagen-activated receptor upstream of the AKT pathway [58]. The

impact of dasatinib on *CDH1*-null cells is described later in the section 'dual DDR2/SRC inhibitors'.

AKT Inhibitors Several allosteric AKT inhibitors, miransertib (ARQ-092), MK-2206, perifosine, and SC66 preferentially inhibit MCF10A *CDH1*-null cells relative to wild-type cells [49]. These synthetic lethal effects were not observed with the ATP-competitive AKT inhibitor ipatasertib, either because of the lower specificity of ATP-pocket binding inhibitors compared to allosteric inhibitors, or the propensity of ATP-competitive AKT inhibitors to paradoxically cause hyperphosphorylation of their targets [59]. Each of the allosteric AKT inhibitors also caused significantly more apoptotic priming [60] and apoptosis in the MCF10A *CDH1*-null cells compared to wild-type cells, indicating their effects are, at least in part, cytotoxic and not just cytostatic [49]. Miransertib and MK-2206 also demonstrated synthetic lethal effects in both *CDH1* isogenic NCI-N87 gastric cancer cells and *CDH1* isogenic mouse-derived gastric and mammary organoids [50].

Miransertib is being assessed for the long-term treatment of Proteus syndrome, an overgrowth disorder caused by a mosaic variant of *AKT1* [61]. A dose of 10 mg/day has been shown to be sufficient to reduce phosphorylated AKT in affected tissue by 50%, a dose that is a fraction of the 30–60 mg/day continuous, maximum tolerated dose in adults [61]. At this dose, miransertib only has mild toxicity, with one patient being reported to take between 10 and 20 mg/day for 5 years and only experiencing minor side effects during that time [62]. It is yet to be determined whether this dose is sufficient to inhibit the growth or promote apoptosis of stage T1a gastric SRCCs. If not, non-continuous dosing regimens would enable significantly higher concentrations to be considered. Notably, one woman with Proteus syndrome who developed low-grade ovarian cancer has been treated successfully with miransertib at 100 mg/day using a week-on, week-off schedule. The treatment led to complete remission from the cancer and was sufficiently well tolerated to be ongoing after 22 months [63].

Phase 1 studies have reported MK-2206 to be well tolerated, with mild-to-moderate rash and nausea the most common adverse events [64–66]. A maximum tolerated dose of 60 mg on alternate days has been established. Notably, dermatological toxicities were not observed at a lower dose of 30 mg [64]. Because of its long half-life (60–80 h), MK-2206 can be administered on an intermittent, weekly schedule [66]. When given once weekly, a maximum tolerated dose of 200 mg/week has been established. This weekly dosing schedule was as well tolerated as the 60 mg alternate-day schedule and reduced the pSer473 AKT signal in tumour biopsies to 50% of baseline levels. As for miransertib, it remains to be determined if a 50% reduction in phosphoAKT is sufficient to eliminate stage T1a SRCCs; however, these phase 1 studies suggest that an effective, tolerable, dosing schedule may be possible for these allosteric AKT inhibitors.

In human gastric tumours, the *AKT3* isoform, but not *AKT1* and *AKT2*, is highly over-expressed in tumours with low *CDH1* expression, raising the possibility that lower systemic toxicity might be achieved by specifically targeting AKT3 [49]. Unfortunately, AKT3-specific inhibitors have not yet been developed. In an

effort to indirectly target AKT3, Bougen-Zhukov et al. analysed the TCGA and GEO gastric cancer RNAseq datasets for genes and signalling pathways that were positively correlated with *AKT3* but not *AKT1* or *AKT2* [49, 50]. Eight statistically significantly enriched Reactome pathways were identified, dominated by ECM-related pathways including non-integrin membrane-ECM interactions, ECM organisation, degradation of the ECM, and collagen degradation [50]. The most highly correlated single gene in both datasets was DDR2, a collagen-activated receptor kinase that is involved in the regulation of cell survival, migration, differentiation, and ECM remodelling [67, 68]. A specific allosteric inhibitor of DDR2 (WRG-028) was synthetic lethal in *CDH1* MCF10A isogenic cells and *Cdh1*-null mouse gastric and mammary gland organoids (Decourtye-Espiard, unpublished results), suggesting the importance of DDR2-AKT3 signalling to *CDH1*-null cells [50].

Dual DDR2/SRC Inhibitors In addition to WRG-028, DDR2 is also inhibited by several FDA-approved receptor tyrosine kinase antagonists, including imatinib, dasatinib, ponatinib, and nilotinib. These four drugs were originally developed to target the BCR-ABL fusion, but were subsequently shown to inhibit several other kinases including DDR2 and SRC [58, 69–71]. Of these four drugs, *CDH1*-null MCF10A cells were more sensitive than wild-type cells to both imatinib and dasatinib, with dasatinib active in the nanomolar range [50]. The sensitivity of *CDH1*-null cells to dasatinib was confirmed in mouse gastric and mammary gland organoids that were isogenic for both *Cdh1* and *Cdh1/Tp53* [50]. Dasatinib was confirmed to reduce AKT signalling in the mouse *Cdh1*-null organoids, supporting the importance of this pathway to *CDH1* synthetic lethality. It is probable that the synthetic lethal effect of dasatinib is driven by its dual inhibition of both DDR2 and the SRC kinase family, with inhibition of both targets reducing survival signalling through the AKT pathway.

The standard dose of dasatinib in chronic myeloid leukaemia has been 100 mg once daily, although more recent studies have successfully used daily doses of 20–50 mg for up to 2 years, with excellent clinical responses and adverse events that were largely only mild to moderate [72, 73]. The acceptable toxicity profiles of dasatinib in the 20–100 mg dose range provide important guidance on experiments that aim to validate the efficacy of dasatinib against stage T1a SRCCs.

Autophagy Inhibitors The autophagy inhibitors chloroquine, hydroxychloroquine, and STF-62247 all preferentially inhibit the growth of *CDH1*-null cell lines, with chloroquine being further validated in *Cdh1*-null mouse gastric organoids [46]. Chloroquine accumulates in lysosomes, inhibiting neoglycolipid metabolism and proteolysis, thus preventing degradation of autolysosomes and blocking autophagy. This drug is historically used for the prophylaxis and treatment of malaria and, more recently, the treatment of systemic lupus erythematosus. Chloroquine is generally very well tolerated; its most common side effects are headache and nausea, occurring in about 10% of patients. However, long-term use is associated with more serious side effects, including retinal, cardiac, cutaneous,

and muscle toxicities. For example, retinal toxicity is observed in up to 20% of patients treated for 20 years [74].

Chloroquine's distinct mechanism of action compared to other chemoprevention candidates suggest that it could be used in combination to increase efficacy, but with less likelihood of causing overlapping toxicities. Chloroquine's effect on *CDH1*-null cells has already been shown to be synergistic with MK-2206 (W. Mitchell, unpublished results) and the sphingosine kinase inhibitor PF-543 [46].

Statins Atorvastatin is an exceptionally well-tolerated lipid-lowering HMG-CoA reductase inhibitor that preferentially inhibits the viability of *CDH1*-null MCF10A and NCI-N87 cells, both alone and as part of synergistic combinations ([34, 45, 46] and W. Mitchell, unpublished results). Like chloroquine, atorvastatin's distinct mechanism of action may enable chemoprevention drug combinations that are more effective and better tolerated than single drugs. However, the concentrations required to reduce *CDH1*-null cell viability in vitro (up to the low mM range) are unlikely to be achievable in vivo through oral delivery, due to the low proportion of atorvastatin (~2%) which isn't protein-bound in blood [75]. Other delivery methods, such as direct application to the gastric mucosa, may still enable statins to play an important role in future HDGC chemoprevention.

Additional Potential Chemoprevention Candidates Brew et al. identified sphingolipid metabolism and signalling as a vulnerability in *CDH1*-null cells [46]. In particular, the sphingosine kinase inhibitor PF-543 showed a strong synthetic lethal effect in both *CDH1*-null MCF10A cells and gastric organoids, although the effect wasn't observed in the NCI-N87 gastric cancer cell line, illustrating the importance of genetic background to the response to this drug [46]. Although PF-543 has not been developed for clinical use, a dual SPHK1/Protein Kinase C antagonist, safingol, and several sphingosine-1 phosphate receptor modulators, have been shown to have good safety profiles in phase 1 trials, supporting closer investigation of these classes of drugs [76, 77].

Drugs active against sporadic, advanced DGCs could also be tested for their ability to preferentially inhibit *CDH1*-null cells. Of particular note are the focal adhesion kinase (FAK) inhibitors PF573228 and defactinib which, between them, have been shown to attenuate AKT signalling, reverse the aberrant morphology in $Cdh1^{-/-}$ $RHOA^{Y42C/+}$ organoids, and abrogate the growth of $Cdh1^{-/-}$ $RHOA^{Y42C/+}$ organoid xenografts [78]. The antagonism of tumour-ECM interactions by FAK inhibitors and integrin inhibitors [79], or other drugs emerging for the treatment of fibrotic diseases, likely represent an important area of ongoing research.

21.8 Minimisation of Drug Side Effects

To be realistically considered for chemoprevention, an effective drug must have a minimal toxicity profile that neither significantly compromises quality of life nor creates additional health risks—a high hurdle for cancer drugs which, in general,

target the fundamental cellular drivers of proliferation and survival. However, three strategies can be employed to mitigate the risk of HDGC chemoprevention candidates being too poorly tolerated to be used over many years: long intervals between repeat administrations, drug dose minimisation, and tissue-specific drug delivery.

The potential for long intervals between consecutive gastric cancer chemoprevention treatments is enabled by the relative indolence of the stage T1a gastric SRCCs. Although the maximum interval cannot yet be absolutely defined, data from both sporadic gastric cancer progression rates and endoscopic surveillance of *CDH1* mutation carriers suggest that early-stage T1a foci are unlikely to progress to ≥stage 2 disease within a 1-year period [2, 7, 80–82]. This suggests that mutation carriers may be able to be safely treated with chemoprevention drugs at yearly intervals.

Drug doses considerably lower than those typically used on advanced solid tumours may be effective for gastric cancer chemoprevention, due to the small average size of the gastric T1a foci, their accessibility to drug, and the heightened sensitivity of *CDH1*-null cells to the selected agents. The average foci diameter (<1 mm) and the absence of desmoplasia at this very early tumour stage ensures a short drug diffusion distance and reduces the likelihood of high interstitial fluid pressure, tumour density, or abnormal vasculature inhibiting drug delivery [83]. Further dose reductions could also be achieved through the use of additive or synergistic drug combinations. For example, drug synergy has been described in *CDH1*-null cells, including for dasatinib/MK2206, PF-543/chloroquine, and PF-543/atorvastatin [46, 50]. The benefits of combinations will be expected to be greatest when the drugs do not have significantly overlapping drug toxicities, although the effect of drug synergy on normal tissue will also need to be carefully evaluated.

Finally, direct-to-tissue drug delivery would reduce the systemic side effects of cancer treatments, greatly increasing tolerability. For stomach tissue, this could be achieved by drug delivery systems that are designed to release drug in a rate-controlled manner only in the acidic environment of the stomach [84]. Combining this controlled release with a gastro-retentive formulation would enable the drug to be retained in the stomach for extended periods [85]. One gastro-retentive formulation that has been well established for the treatment of gastroesophageal reflux disease is the biocompatible polysaccharide alginate, which forms low-density raft-like structures that float on the gastric fluid [86]. An alternative formulation would be polymers such as polyacrylic acid (Carbopol®) that prolong gastric residence times by forming an adhesion to the gastric mucosa [87, 88]. Mucoadhesive polymer–drug conjugates could conceivably be applied systematically to the mucosal surface at the time of routine endoscopic examination.

Tissue-specific delivery of chemoprevention drugs to the breast may also be possible using a transdermal route and lipophilic formulations and compounds [89]. Transdermal delivery of an active metabolite of the breast cancer chemoprevention drug tamoxifen showed that pharmacologically effective breast concentrations could be achieved with low systemic exposure [90, 91]. Importantly, recent evidence also suggests that transdermal delivery can lead to anatomical drug

distribution patterns in the breast that are comparable to those achieved with oral drug delivery [92].

21.9 Future Development Path

Beyond *CDH1* isogenic cell line and organoid models, candidate chemoprevention compounds will need to be validated in mouse gastric cancer models, followed by human tissue studies and prospective clinical trials. One promising inducible mouse model is the CD44-cre/$Cdh1^{fl/fl}$/$Tp53^{fl/fl}$ mouse which develops stage 3 gastric cancer within 3 months of induction (Decourtye-Espiard, unpublished data). This rapid timeline for disease progression and its simple genetic background will facilitate the rapid triage of candidates prior to human studies. Validation in this model would be enhanced by the use of scRNAseq to determine drug efficacy in each cell sub-population within the tumour [93].

It will be important to determine early whether chemoprevention candidates are likely to cause apoptosis of *CDH1*-null cells in vivo at concentrations that are compatible with safe, well-tolerated use. For example, as noted earlier, concentrations of the AKT inhibitors miransertib and MK-2206 that are well tolerated in vivo still inhibit AKT phosphorylation by ~50% [61, 66]. Therefore, demonstration that these drugs are able to cause apoptosis of *CDH1*-null cells in pre-clinical models at concentrations that reduce AKT signalling by 50% would support clinical testing.

Unfortunately, clinical trials will be complicated by our current inability to quantify the size and number of stage T1a gastric foci in vivo. As a result, before any formal chemoprevention trials can be considered, drug efficacy in humans will need to be first assessed in cohorts of *CDH1* mutation carriers who have decided to proceed with a prophylactic gastrectomy, but are amenable to drug treatment prior to surgery. In these studies, the goal would be to obtain immunohistochemical evidence that the stage T1a foci in each patient's stomach are sensitive to the drug(s) and showing evidence of apoptosis and regression. This would be complemented by the parallel identification of surrogate markers of drug activity that can be used on biopsies of normal gastric tissue to indicate that there has been sufficient drug exposure to drive the apoptosis of stage T1a SRCCs. Subsequent prospective clinical trials could then be considered in a staged manner, beginning with *CDH1* mutation carriers who wish to delay—but not completely bypass—prophylactic gastrectomy, before proceeding to long-term trials in which chemoprevention is an adjunct to annual surveillance. Since many of the drug candidates identified to date are also synthetic lethal in *CDH1*-null breast cells and mouse mammary gland organoids, a similar path to breast cancer chemoprevention is possible.

21.10 Conclusion

Transient downregulation of E-cadherin is part of normal epithelial cell programming. Consequently, cells with permanent, inactivating *CDH1* mutations remain robust—protected by highly evolved homeostatic mechanisms. However, homeostasis is not limitless in its protection. E-cadherin-null cells undergoing important functional changes including to the organisation of the plasma membrane, the efficiency of vesicle trafficking, and its interactions with the ECM that expose vulnerabilities that can be exploited with drugs. These vulnerabilities, combined with the small size, relative indolence, and genetic homogeneity of the stage T1a gastric SRCC, suggest that chemoprevention of HDGC is achievable, providing the means for HDGC families to avoid the complications of prophylactic surgery and reduce the risks associated with long-term surveillance.

Acknowledgements We thank Allan Gamble and Jessica Fairhall (School of Pharmacy, University of Otago) for discussions on stomach drug delivery systems. Most of all, we thank the families affected by HDGC for their support and inspiration.

References

1. Guilford P, Hopkins J, Harraway J et al (1998) E-cadherin germline mutations in familial gastric cancer. Nature 392:402–405
2. Blair VR, McLeod M, Carneiro F et al (2020) Hereditary diffuse gastric cancer: updated clinical practice guidelines. Lancet Oncol 21(8):e386–ee97. https://doi.org/10.1016/s1470-2045(20)30219-9
3. Pharoah PD, Guilford P, Caldas C et al (2001) Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. Gastroenterology 121(6):1348–1353. https://doi.org/10.1053/gast.2001.29611
4. Xicola RM, Li S, Rodriguez N et al (2019) Clinical features and cancer risk in families with pathogenic CDH1 variants irrespective of clinical criteria. J Med Genet 56(12):838–843. https://doi.org/10.1136/jmedgenet-2019-105991
5. Roberts ME, Ranola JMO, Marshall ML et al (2019) Comparison of CDH1 penetrance estimates in clinically ascertained families vs families ascertained for multiple gastric cancers. JAMA Oncol 5(9):1325–1331. https://doi.org/10.1001/jamaoncol.2019.1208
6. Hansford S, Kaurah P, Li-Chang H et al (2015) Hereditary diffuse gastric cancer syndrome: CDH1 mutations and beyond. JAMA Oncol 1:23–32
7. van Dieren JM, Kodach LL, den Hartog P et al (2020) Gastroscopic surveillance with targeted biopsies compared with random biopsies in CDH1 mutation carriers. Endoscopy 52(10):839–846. https://doi.org/10.1055/a-1157-8678
8. Heiniger L, Butow PN, Coll J et al (2015) Long-term outcomes of risk-reducing surgery in unaffected women at increased familial risk of breast and/or ovarian cancer. Familial Cancer 14(1):105–115. https://doi.org/10.1007/s10689-014-9759-7
9. Zhang X, Oliveri JM, Paskett ED (2020) Features, predictors, and treatment of breast cancer-related lymphedema. Curr Breast Cancer Rep 12(4):244–254. https://doi.org/10.1007/s12609-020-00381-0
10. Guilford P, Humar B, Blair V (2010) Hereditary diffuse gastric cancer: translation of CDH1 germline mutations into clinical practice. Gastric Cancer 13(1):1–10. https://doi.org/10.1007/s10120-009-0531-x

11. Charlton A, Blair V, Shaw D et al (2004) Hereditary diffuse gastric cancer: predominance of multiple foci of signet ring cell carcinoma in distal stomach and transitional zone. Gut 53(6): 814–820
12. Humar B, Blair V, Charlton A et al (2009) E-cadherin deficiency initiates gastric signet-ring cell carcinoma in mice and man. Cancer Res 69(5):2050–2056
13. Grady WM, Willis J, Guilford PJ et al (2000) Methylation of the CDH1 promoter as the second genetic hit in hereditary diffuse gastric cancer. Nat Genet 26(1):16–17
14. Humar B, Guilford P (2009) Hereditary diffuse gastric cancer: a manifestation of lost cell polarity. Cancer Sci 100(7):1151–1157. https://doi.org/10.1111/j.1349-7006.2009.01163.x
15. Gloerich M, Bianchini JM, Siemers KA et al (2017) Cell division orientation is coupled to cell-cell adhesion by the E-cadherin/LGN complex. Nat Commun 8:13996. https://doi.org/10.1038/ncomms13996
16. Bulgakova NA, Klapholz B, Brown NH (2012) Cell adhesion in Drosophila: versatility of cadherin and integrin complexes during development. Curr Opin Cell Biol 24(5):702–712. https://doi.org/10.1016/j.ceb.2012.07.006
17. Le Borgne R, Bellaiche Y, Schweisguth F (2002) Drosophila E-cadherin regulates the orientation of asymmetric cell division in the sensory organ lineage. Curr Biol 12(2):95–104
18. den Elzen N, Buttery CV, Maddugoda MP et al (2009) Cadherin adhesion receptors orient the mitotic spindle during symmetric cell division in mammalian epithelia. Mol Biol Cell 20(16): 3740–3750. https://doi.org/10.1091/mbc.E09-01-0023
19. Humar B, Guilford P (2008) Hereditary diffuse gastric cancer and lost cell polarity: a short path to cancer. Future Oncol 4(2):229–239. https://doi.org/10.2217/14796694.4.2.229
20. Humar B, Fukuzawa R, Blair V et al (2007) Destabilized adhesion in the gastric proliferative zone and c-Src kinase activation mark the development of early diffuse gastric cancer. Cancer Res 67(6):2480–2489
21. Kluijt I, Siemerink EJ, Ausems MG et al (2012) CDH1-related hereditary diffuse gastric cancer syndrome: clinical variations and implications for counseling. Int J Cancer 131(2):367–376. https://doi.org/10.1002/ijc.26398
22. Tanis PJ, Ten Kate FJ, van Lanschot JJ (2008) No mucosal escape of signet-ring cell carcinoma for ten years in a patient with familial aggregation of gastric cancer. J Surg Oncol 97(3): 294–296
23. Seidlitz T, Chen YT, Uhlemann H et al (2019) Mouse models of human gastric cancer subtypes with stomach-specific CreERT2-mediated pathway alterations. Gastroenterology 157(6): 1599–614.e2. https://doi.org/10.1053/j.gastro.2019.09.026
24. Mirandola S, Pellini F, Granuzzo E et al (2019) Multidisciplinary management of CDH1 germinal mutation and prophylactic management hereditary lobular breast cancer: a case report. Int J Surg Case Rep 58:92–95. https://doi.org/10.1016/j.ijscr.2019.03.053
25. Corso G, Figueiredo J, La Vecchia C et al (2018) Hereditary lobular breast cancer with an emphasis on E-cadherin genetic defect. J Med Genet 55(7):431–441. https://doi.org/10.1136/jmedgenet-2018-105337
26. Blair VR (2008) Hereditary diffuse gastric cancer: of mice and man. University of Auckland, Auckland
27. Abreu-Blanco MT, Watts JJ, Verboon JM et al (2012) Cytoskeleton responses in wound repair. Cell Mol Life Sci 69(15):2469–2483. https://doi.org/10.1007/s00018-012-0928-2
28. Oliveira C, Senz J, Kaurah P et al (2009) Germline CDH1 deletions in hereditary diffuse gastric cancer families. Hum Mol Genet 18(9):1545–1555
29. Debnath J, Muthuswamy SK, Brugge JS (2003) Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures. Methods 30(3):256–268
30. Soule HD, Maloney TM, Wolman SR et al (1990) Isolation and characterization of a spontaneously immortalized human breast epithelial cell line, MCF-10. Cancer Res 50(18):6075–6086

31. Chen A, Beetham H, Black MA et al (2014) E-cadherin loss alters cytoskeletal organization and adhesion in non-malignant breast cells but is insufficient to induce an epithelial-mesenchymal transition. BMC Cancer 14(1):552. https://doi.org/10.1186/1471-2407-14-552
32. Weeks SE, Metge BJ, Samant RS (2019) The nucleolus: a central response hub for the stressors that drive cancer progression. Cell Mol Life Sci 76(22):4511–4524. https://doi.org/10.1007/s00018-019-03231-0
33. Telford B, Chen A, Beetham H et al (2015) Synthetic lethal screens identify vulnerabilities in GPCR signalling and cytoskeletal organisation in E-cadherin-deficient cells. Mol Cancer Ther 14:1213–1223
34. Godwin TD, Kelly ST, Brew TP et al (2019) E-cadherin-deficient cells have synthetic lethal vulnerabilities in plasma membrane organisation, dynamics and function. Gastric Cancer 22(2):273–286. https://doi.org/10.1007/s10120-018-0859-1
35. Kenworthy AK, Schmieder SS, Raghunathan K et al (2021) Cholera toxin as a probe for membrane biology. Toxins (Basel) 13(8):543. https://doi.org/10.3390/toxins13080543
36. De Santis G, Miotti S, Mazzi M et al (2009) E-cadherin directly contributes to PI3K/AKT activation by engaging the PI3K-p85 regulatory subunit to adherens junctions of ovarian carcinoma cells. Oncogene 28(9):1206–1217. https://doi.org/10.1038/onc.2008.470
37. Pece S, Chiariello M, Murga C et al (1999) Activation of the protein kinase Akt/PKB by the formation of E-cadherin-mediated cell-cell junctions. Evidence for the association of phosphatidylinositol 3-kinase with the E-cadherin adhesion complex. J Biol Chem 274(27):19347–19351
38. Kovacs EM, Ali RG, McCormack AJ et al (2002) E-cadherin homophilic ligation directly signals through Rac and phosphatidylinositol 3-kinase to regulate adhesive contacts. J Biol Chem 277(8):6708–6718. https://doi.org/10.1074/jbc.M109640200
39. Benham-Pyle BW, Pruitt BL, Nelson WJ (2015) Cell adhesion. Mechanical strain induces E-cadherin-dependent Yap1 and beta-catenin activation to drive cell cycle entry. Science 348(6238):1024–1027. https://doi.org/10.1126/science.aaa4559
40. Kim NG, Koh E, Chen X et al (2011) E-cadherin mediates contact inhibition of proliferation through Hippo signaling-pathway components. Proc Natl Acad Sci U S A 108(29):11930–11935. https://doi.org/10.1073/pnas.1103345108
41. Honigmann A, Pralle A (2016) Compartmentalization of the cell membrane. J Mol Biol 428(24 Pt A):4739–4748. https://doi.org/10.1016/j.jmb.2016.09.022
42. Head BP, Patel HH, Insel PA (2014) Interaction of membrane/lipid rafts with the cytoskeleton: impact on signaling and function: membrane/lipid rafts, mediators of cytoskeletal arrangement and cell signaling. Biochim Biophys Acta 1838(2):532–545. https://doi.org/10.1016/j.bbamem.2013.07.018
43. Villar VA, Cuevas S, Zheng X et al (2016) Localization and signaling of GPCRs in lipid rafts. Methods Cell Biol 132:3–23. https://doi.org/10.1016/bs.mcb.2015.11.008
44. Delos Santos RC, Garay C, Antonescu CN (2015) Charming neighborhoods on the cell surface: plasma membrane microdomains regulate receptor tyrosine kinase signaling. Cell Signal 27(10):1963–1976. https://doi.org/10.1016/j.cellsig.2015.07.004
45. Single AB (2017) Synergistic synthetic lethal drug combinations for E-cadherin-deficient cancers. University of Otago, Dunedin
46. Brew T, Bougen-Zhukov N, Mitchell W et al (2021) Loss of E-cadherin leads to druggable vulnerabilities in sphingolipid metabolism and vesicle trafficking. Cancers (Basel) 14(1):102. https://doi.org/10.3390/cancers14010102
47. Beetham H, Chen A, Telford B et al (2019) A high-throughput screen to identify novel synthetic lethal compounds for the treatment of E-cadherin-deficient cells. Sci Rep 9(1):12511. https://doi.org/10.1038/s41598-019-48929-0
48. Decourtye-Espiard L, Bougen-Zhukov N, Godwin T et al (2021) E-cadherin-deficient epithelial cells are sensitive to HDAC inhibitors. Cancers (Basel) 14(1):175. https://doi.org/10.3390/cancers14010175

49. Bougen-Zhukov N, Nouri Y, Godwin T et al (2019) Allosteric AKT inhibitors target synthetic lethal vulnerabilities in E-cadherin-deficient cells. Cancers (Basel) 11(9):1359. https://doi.org/10.3390/cancers11091359
50. Bougen-Zhukov N, Decourtye-Espiard L, Redpath K et al (2021) Targeting of AKT3-associated genes with dasatinib as a strategy to treat E-cadherin-deficient cancers. Cancer 14(7):1609
51. Shah P, Gau Y, Sabnis G (2014) Histone deacetylase inhibitor entinostat reverses epithelial to mesenchymal transition of breast cancer cells by reversing the repression of E-cadherin. Breast Cancer Res Treat 143(1):99–111. https://doi.org/10.1007/s10549-013-2784-7
52. Nagathihalli NS, Massion PP, Gonzalez AL et al (2012) Smoking induces epithelial-to-mesenchymal transition in non-small cell lung cancer through HDAC-mediated downregulation of E-cadherin. Mol Cancer Ther 11(11):2362–2372. https://doi.org/10.1158/1535-7163.Mct-12-0107
53. Yardley DA, Ismail-Khan RR, Melichar B et al (2013) Randomized phase II, double-blind, placebo-controlled study of exemestane with or without entinostat in postmenopausal women with locally recurrent or metastatic estrogen receptor-positive breast cancer progressing on treatment with a nonsteroidal aromatase inhibitor. J Clin Oncol 31(17):2128–2135. https://doi.org/10.1200/jco.2012.43.7251
54. Masuda N, Tamura K, Yasojima H et al (2021) Phase 1 trial of entinostat as monotherapy and combined with exemestane in Japanese patients with hormone receptor-positive advanced breast cancer. BMC Cancer 21(1):1269. https://doi.org/10.1186/s12885-021-08973-4
55. Martellucci S, Clementi L, Sabetta S et al (2020) Src family kinases as therapeutic targets in advanced solid tumors: what we have learned so far. Cancers (Basel) 12(6):1448. https://doi.org/10.3390/cancers12061448
56. Lombardo LJ, Lee FY, Chen P et al (2004) Discovery of N-(2-chloro-6-methyl- phenyl)-2-(6-(4-(2-hydroxyethyl)- piperazin-1-yl)-2-methylpyrimidin-4- ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. J Med Chem 47(27):6658–6661. https://doi.org/10.1021/jm049486a
57. Lindauer M, Hochhaus A (2018) Dasatinib. Recent Results Cancer Res 212:29–68. https://doi.org/10.1007/978-3-319-91439-8_2
58. Denny WA, Flanagan JU (2021) Inhibitors of discoidin domain receptor (DDR) kinases for cancer and inflammation. Biomolecules 11(11):1671. https://doi.org/10.3390/biom11111671
59. Okuzumi T, Fiedler D, Zhang C et al (2009) Inhibitor hijacking of Akt activation. Nat Chem Biol 5(7):484–493. https://doi.org/10.1038/nchembio.183
60. Ryan JA, Brunelle JK, Letai A (2010) Heightened mitochondrial priming is the basis for apoptotic hypersensitivity of CD4+ CD8+ thymocytes. Proc Natl Acad Sci U S A 107(29):12895–12900. https://doi.org/10.1073/pnas.0914878107
61. Keppler-Noreuil KM, Sapp JC, Lindhurst MJ et al (2019) Pharmacodynamic Study of Miransertib in Individuals with Proteus Syndrome. Am J Hum Genet 104(3):484–491. https://doi.org/10.1016/j.ajhg.2019.01.015
62. Ours CA, Sapp JC, Hodges MB et al (2021) Case report: five-year experience of AKT inhibition with miransertib (MK-7075) in an individual with Proteus syndrome. Cold Spring Harb Mol Case Stud 7(6):a006134. https://doi.org/10.1101/mcs.a006134
63. Leoni C, Gullo G, Resta N et al (2019) First evidence of a therapeutic effect of miransertib in a teenager with Proteus syndrome and ovarian carcinoma. Am J Med Genet A 179(7):1319–1324. https://doi.org/10.1002/ajmg.a.61160
64. Yap TA, Yan L, Patnaik A et al (2011) First-in-man clinical trial of the oral pan-AKT inhibitor MK-2206 in patients with advanced solid tumors. J Clin Oncol 29(35):4688–4695. https://doi.org/10.1200/jco.2011.35.5263
65. Doi T, Tamura K, Tanabe Y et al (2015) Phase 1 pharmacokinetic study of the oral pan-AKT inhibitor MK-2206 in Japanese patients with advanced solid tumors. Cancer Chemother Pharmacol 76(2):409-16. https://doi.org/10.1007/s00280-015-2810-z

66. Yap TA, Yan L, Patnaik A et al (2014) Interrogating two schedules of the AKT inhibitor MK-2206 in patients with advanced solid tumors incorporating novel pharmacodynamic and functional imaging biomarkers. Clin Cancer Res 20(22):5672–5685. https://doi.org/10.1158/1078-0432.Ccr-14-0868
67. Leitinger B (2014) Discoidin domain receptor functions in physiological and pathological conditions. Int Rev Cell Mol Biol 310:39–87. https://doi.org/10.1016/b978-0-12-800180-6.00002-5
68. Berestjuk I, Lecacheur M, Carminati A et al (2022) Targeting discoidin domain receptors DDR1 and DDR2 overcomes matrix-mediated tumor cell adaptation and tolerance to BRAF-targeted therapy in melanoma. EMBO Mol Med 14(2):e11814. https://doi.org/10.15252/emmm.201911814
69. Rix U, Hantschel O, Dürnberger G et al (2007) Chemical proteomic profiles of the BCR-ABL inhibitors imatinib, nilotinib, and dasatinib reveal novel kinase and nonkinase targets. Blood 110(12):4055–4063. https://doi.org/10.1182/blood-2007-07-102061
70. Day E, Waters B, Spiegel K et al (2008) Inhibition of collagen-induced discoidin domain receptor 1 and 2 activation by imatinib, nilotinib and dasatinib. Eur J Pharmacol 599(1–3):44–53. https://doi.org/10.1016/j.ejphar.2008.10.014
71. Canning P, Tan L, Chu K et al (2014) Structural mechanisms determining inhibition of the collagen receptor DDR1 by selective and multi-targeted type II kinase inhibitors. J Mol Biol 426(13):2457–2470. https://doi.org/10.1016/j.jmb.2014.04.014
72. Murai K, Ureshino H, Kumagai T et al (2021) Low-dose dasatinib in older patients with chronic myeloid leukaemia in chronic phase (DAVLEC): a single-arm, multicentre, phase 2 trial. Lancet Haematol 8(12):e902–ee11. https://doi.org/10.1016/s2352-3026(21)00333-1
73. Naqvi K, Jabbour E, Skinner J et al (2020) Long-term follow-up of lower dose dasatinib (50 mg daily) as frontline therapy in newly diagnosed chronic-phase chronic myeloid leukemia. Cancer 126(1):67–75. https://doi.org/10.1002/cncr.32504
74. Muller R (2021) Systemic toxicity of chloroquine and hydroxychloroquine: prevalence, mechanisms, risk factors, prognostic and screening possibilities. Rheumatol Int 41(7):1189–1202. https://doi.org/10.1007/s00296-021-04868-6
75. Björkhem-Bergman L, Lindh JD, Bergman P (2011) What is a relevant statin concentration in cell experiments claiming pleiotropic effects? Br J Clin Pharmacol 72(1):164–165. https://doi.org/10.1111/j.1365-2125.2011.03907.x
76. Dickson MA, Carvajal RD, Merrill AH Jr et al (2011) A phase I clinical trial of safingol in combination with cisplatin in advanced solid tumors. Clin Cancer Res 17(8):2484–2492. https://doi.org/10.1158/1078-0432.Ccr-10-2323
77. Companioni O, Mir C, Garcia-Mayea Y et al (2021) Targeting sphingolipids for cancer therapy. Front Oncol 11:745092. https://doi.org/10.3389/fonc.2021.745092
78. Zhang H, Schaefer A, Wang Y et al (2020) Gain-of-function RHOA mutations promote focal adhesion kinase activation and dependency in diffuse gastric cancer. Cancer Discov 10(2):288–305. https://doi.org/10.1158/2159-8290.Cd-19-0811
79. Slack RJ, Macdonald SJF, Roper JA et al (2022) Emerging therapeutic opportunities for integrin inhibitors. Nat Rev Drug Discov 21(1):60–78. https://doi.org/10.1038/s41573-021-00284-4
80. Murakami K, Matsubara H (2018) Chronology of gastrointestinal cancer. Surg Today 48(4):365–370. https://doi.org/10.1007/s00595-017-1574-y
81. Mi EZ, Mi EZ, di Pietro M et al (2018) Comparative study of endoscopic surveillance in hereditary diffuse gastric cancer according to CDH1 mutation status. Gastrointest Endosc 87(2):408–418. https://doi.org/10.1016/j.gie.2017.06.028
82. Gamble LA, Davis JL (2022) Surveillance and surgical considerations in hereditary diffuse gastric cancer. Gastrointest Endosc Clin N Am 32(1):163–175. https://doi.org/10.1016/j.giec.2021.08.009
83. Dewhirst MW, Secomb TW (2017) Transport of drugs from blood vessels to tumour tissue. Nat Rev 17(12):738–750. https://doi.org/10.1038/nrc.2017.93

84. Zhu YJ, Chen F (2015) pH-responsive drug-delivery systems. Chem Asian J 10(2):284–305. https://doi.org/10.1002/asia.201402715
85. Das S, Kaur S, Rai VK (2021) Gastro-retentive drug delivery systems: a recent update on clinical pertinence and drug delivery. Drug Deliv Transl Res 11(5):1849–1877. https://doi.org/10.1007/s13346-020-00875-5
86. Kim HM (2016) Raft formation of sodium alginate in the stomach. J Neurogastroenterol Motil 22(4):705–706. https://doi.org/10.5056/jnm16068
87. Tao Y, Lu Y, Sun Y et al (2009) Development of mucoadhesive microspheres of acyclovir with enhanced bioavailability. Int J Pharm 378(1–2):30–36. https://doi.org/10.1016/j.ijpharm.2009.05.025
88. Laffleur F, Bernkop-Schnürch A (2013) Strategies for improving mucosal drug delivery. Nanomedicine (Lond) 8(12):2061–2075. https://doi.org/10.2217/nnm.13.178
89. Neupane R, Boddu SHS, Abou-Dahech MS et al (2021) Transdermal delivery of chemotherapeutics: strategies, requirements, and opportunities. Pharmaceutics 13(7):960. https://doi.org/10.3390/pharmaceutics13070960
90. Rouanet P, Linares-Cruz G, Dravet F et al (2005) Neoadjuvant percutaneous 4-hydroxytamoxifen decreases breast tumoral cell proliferation: a prospective controlled randomized study comparing three doses of 4-hydroxytamoxifen gel to oral tamoxifen. J Clin Oncol 23(13):2980–2987. https://doi.org/10.1200/jco.2005.06.064
91. Lee O, Page K, Ivancic D et al (2014) A randomized phase II presurgical trial of transdermal 4-hydroxytamoxifen gel versus oral tamoxifen in women with ductal carcinoma in situ of the breast. Clin Cancer Res 20(14):3672–3682. https://doi.org/10.1158/1078-0432.Ccr-13-3045
92. Lee O, Pilewskie M, Karlan S et al (2021) Local transdermal delivery of telapristone acetate through breast skin, compared with oral treatment: a randomized double-blind, placebo-controlled phase II trial. Clin Pharmacol Ther 109(3):728–738. https://doi.org/10.1002/cpt.2041
93. Fustero-Torre C, Jiménez-Santos MJ, García-Martín S et al (2021) Beyondcell: targeting cancer therapeutic heterogeneity in single-cell RNA-seq data. Genome Med 13(1):187. https://doi.org/10.1186/s13073-021-01001-x

Malformations and Malformative Syndromes Associated with *CDH1*

22

Roseline Vibert, Jamal Ghoumid, and Patrick R. Benusiglio

Abstract

Malformative syndromes due to *CDH1* pathogenic variants (PV) include non-syndromic cleft lip or palate (CLP) and the blepharocheilodontic syndrome (BCDS). *CDH1* was initially known as a diffuse gastric cancer (DGC) and lobular breast cancer (LBC) susceptibility gene. Subsequently, pathogenic variants (PV) were reported in individuals with isolated or familial CLP, not necessarily in association with DGC or LBC. Even more recently, *CDH1* variants, but also of its partner *CTNND1*, were identified as the cause of BCDS. This rare disease is characterized by CLP, hypertelorism, and eyelid, dental, and hair abnormalities. There is no obvious genotype–phenotype correlation. However, most BCDS-causing variants are missense and splice site and are located in two mutational hotspots. The prevalence of malformative syndromes due to *CDH1* is not known.

In this chapter, we will describe the association of *CDH1* with CLP and BCDS. We will also discuss the physiopathology of these associations and discuss potential explanations for the variety of observed phenotypes.

22.1 Introduction

Beyond their involvement in predisposition to diffuse gastric (DGC) and lobular breast cancer (LBC), *CDH1* pathogenic variants (PV) are also implicated in birth defects and malformative syndromes.

R. Vibert · P. R. Benusiglio (✉)
UF d'Oncogénétique Clinique, Département de Génétique Médicale et Institut Universitaire de Cancérologie, Hôpital Pitié-Salpêtrière, AP-HP.Sorbonne Université, Paris, France
e-mail: patrick.benusiglio@aphp.fr

J. Ghoumid
Université de Lille, CHU Lille, Clinique de génétique Guy Fontaine, Lille, France

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_22

Since the first description in 1998 of germline loss-of-function variants in the *CDH1* gene involved in hereditary diffuse gastric cancer (HDGC), cleft lip/palate (CLP) has been described in PV carriers. The first families carrying *CDH1* PV with CLP were described in 2006, and since then, several studies have highlighted the involvement of *CDH1* variants in CLP. More recently, patients with a rare malformative syndrome called blepharocheilodontic syndrome (BCDS) were shown to carry *CDH1* variants.

In this chapter, we will describe the association of *CDH1* with CLP and BCDS. We will also discuss the physiopathology of these associations and explore an emerging genotype–phenotype correlation.

22.2 *CDH1* Association with Cleft Lip/Palate

22.2.1 Clinical Description of CLP

CLP is one of the commonest congenital malformations with a variable incidence in different populations, ranging from 1/700 to 1/1000 newborns [1]. The prevalence is reportedly higher in Asians and lower in Africans [2, 3]. CLP is of variable severity. Defects sometimes extend beyond lip and palate, involving for example the forehead. They severely impact primary functions such as feeding, speech, hearing, and often cause psychological distress, requiring long-term multidisciplinary management. Almost 70% of these malformations are non-syndromic [1].

22.2.2 Genetic Causes of CLP

The etiology of non-syndromic CLP remains largely unknown. The increased incidence rate of clefts in newborns from affected families suggests a crucial role of genetic factors in CLP [4]. Genome-wide association studies (GWAS) have identified several genetic loci involved in the etiology of non-syndromic CLP and common nucleotide variants [5–7]. Whole-exome sequencing (WES) in family studies identified rare variants associated with non-syndromic CLP, notably in *CDH1* and several other genes with a role in epithelium development and morphogenesis [8, 9]. The involvement of E-cadherin dysfunction in non-syndromic CLP therefore makes perfect biological sense since it is overexpressed during critical stages of lip and palate development.

22.2.3 A Literature Review of *CDH1* Association with CLP

The first description of an association between CLP and *CDH1* PV was reported in 2006 with the identification of two different *CDH1* splice site variants affecting the extracellular domain of E-cadherin in two HDGC Caucasian families with CLP [10]. The authors also showed that E-cadherin was highly expressed during the 4th

and 5th weeks of development in the frontonasal prominence and in the lateral and medial nasal prominences of embryos during the 6th week supporting this association.

Further studies reported additional families with *CDH1* variants associating HDGC and CLP [11–13]. Notably, Kluijt et al. described seven individuals with CLP in three HDGC Caucasian families [12]. In 2013, Benusiglio et al. reported the case of a patient from Southeast Asian descent with CLP who developed diffuse gastric cancer [11]. She carried a *CDH1* splice site PV. Finally, Obemair et al. reported two additional *CDH1* splice site variants in two families with histories of DGC [13].

CLP was also reported in *CDH1* families without DGC [8, 14–18]. Particularly, Vogelaar et al. identified three *CDH1* missense variants in 4 individuals from a cohort of 81 Caucasian children (5%) with non-syndromic CLP [18]. In 2014, Bureau et al. identified, through WES of 55 multiplex cleft families, a common nonsense variant of *CDH1* in three distant relatives of an Indian family affected by non-syndromic CLP [8].

Cox et al. analyzed 209 individuals from 72 multigenerational families with Mendelian transmission of non-syndromic CLP by WES and found 20 pathogenic or likely PV in genes regulating epithelial cadherin–catenin complex assembly, including variants in *CDH1* [15]. They replicated their results in a second validation cohort of 497 individuals from 444 small families (single and familial case subjects). In total, six likely pathogenic *CDH1* variants were identified, two in the discovery cohort (2.8%) and four in the replication cohort (0.9%). Only one carrier had a family history of gastric cancer.

Additionally, Du et al. described a *CDH1* missense variant segregating in a four-generation Chinese family with autosomal dominant non-syndromic CLP identified by WES [16]. They showed that this variant resulted in decreased E-cadherin dimerization.

Overall, *CDH1* mutations have been reported in CLP patients with and without a DGC family history.

The variants reported in these studies, and others of lesser importance, are listed in Table 22.1. Although more than 30 are reported to be associated with CLP, not all are classified as pathogenic/likely pathogenic as reported in ClinVar or according to the ACMG criteria [19, 20].

22.3 *CDH1* Association with Blepharocheilodontic Syndrome (BCDS)

BCDS is a rare syndrome combining CLP, eyelid malformations, and dental anomalies. It has recently been associated with *CDH1*, thus expanding the phenotypic spectrum of *CDH1*-related anomalies.

Table 22.1 *CDH1* variants reported as associated to clefts of lip and palate

Domain	Mutation	Amino acid change	Location	Type	CL/P cases	Cancer history	References	Clinvar	Selvanathan [19] (classification by ACMG criteria)
Signal/ propeptide	c.88C>A[a]	p.(Pro30Thr)	Exon 2	Missense	2		[18]	B***	
	c.337A>G	p.(Lys113Glu)	Exon 3	Missense	1		[18]	VUS**	
	c.387+5G>A	p.?	Intron 3	Splice site	1		[8]	B***	
Extracellular	c.468G>C	p.(Trp156Cys)	Exon 4	Missense	3		[16]	NA	LP
	c.489C>A	p.(Cys163*)	Exon 4	Nonsense	1 untested (≤50% risk)	3 GC; 1 BC	[12]	P***	P
	c.531+2T>A	p.?	Intron 4	Splice site	4	3 DGC; 1GC	[10]	P	LP
	c.531+3A>G	p.?	Intron 4	Splice site	1		[18]	VUS**	
	c.532–18C>T[a]	p.?	Intron 4	Splice site	1		[18]	B/ LB**	
	c.687+1G>A	p.?	Intron 5	Splice site	3	5 DGC	[13]	LP***	
	c.752C>T	p.(Thr251Met)	Exon 6	Missense	1		[15]	VUS**	LP
	c.760G>A[b]	p.(Asp254Asn)	Exon 6	Missense	8+1		[8, 15]	VUS*	P
	c.832+1G>T	p.?	Intron 6	Splice site	1	1 DGC	[11]	P***	LP
	c.895G>A	p.(Ala299Thr)	Exon 7	Missense	1		[19]	VUS*	LP
	c.1023T>G[a]	p.(Tyr341*)	Exon 8	Nonsense	2		[8]	P***	LP
	c.1108G>T	p.(Asp370Tyr)	Exon 8	Missense	1		[18]	NA	P
	c.1135_1137+5delins5	NA	Exon 8	Splice site	1	4 GC	[12]	NA	
	c.1137G>A	p.(Thr379=)	Exon 8	Splice site	1	2 DGC; 1GC	[10]	P***	P

	c.1235T>C	p.(Val412Ala)	Exon 9	Missense	1		[17]	VUS*	LP
	c.1273G>A	p.(Val425Ile)	Exon 9	Missense	1		[17]	CIP	
	c.1404delC	p.(Thr468Thrfs*13)	Exon 10	Frameshift	3	9 GC; 1 BC	[12]	NA	LP
	c.1489G>A	p.(Glu497Lys)	Exon 10	Missense	1		[15]	VUS**	LP
	c.1565C>T	p.(Thr522Ile)	Exon 10	Missense	1		[17]	CIP	LP
	c.1711+1G>C	p.?	Intron 11	Splice site	1	HDGC	[13]	LP***	LP
	c.1766A>T	p.(Asn589Ile)	Exon 12	Missense	1		[15]	LP	LP
	c.1888C>G	p.(Leu630Val)	Exon 12	Missense	1		[17]	B***	
	c.1926C>A	p.(Tyr642*)	Exon 12	Nonsense	1		[19]	VUS	LP
Transmembrane domain	c.2143G>T	p.(Gly715*)	Exon 13	Nonsense	3		[8]	VUS	P
	c.2143G>A	p.(Gly715Arg)	Exon 13	Missense	NA		[13]	NA	LP
Cytoplasmic domain	c.2351G>A	p.(Arg784His)	Exon 15	Missense	3		[8]	VUS**	LP
	c.2413G>A[a]	p.(Asp805Asn)	Exon 15	Missense	1		[18]	B***	
	c.2426_2427del	p.(Asn809Ilefs*3)	Exon 15	Frameshift	4		[15]	LP	LP
	c.2439+10C>T	p.?	Intron 15	Splice site	1		[18]	B/LB**	
	c.2440–6_2440-4del	p.?	Intron 15	Splice site	1		[18]	NA	

[a]Variants previously identified in HDGC families

[b]Variant also identified in families with blepharocheilodontic syndrome

GC gastric cancer, *BC* breast cancer, *DGC* diffuse gastric cancer, *HDGC* hereditary diffuse gastric cancer, *B* Benign, *LB* likely benign, *VUS* variant of unknown significance, *LP* likely pathogenic, *P* pathogenic, *CIP* conflicting interpretations of pathogenicity; ClinVar: review status: "*": criteria provided, single submitter, "**": criteria provided, multiple submitters, no conflicts, "***": reviewed by expert panel; otherwise: no assertion provided

22.3.1 Clinical Description of BCDS

This syndrome was first called "Elschnig syndrome," referring to his first description of a patient with palpebral fissures, ectropion of the lower eyelids, variable hypertelorism, and CLP in 1912 [21]. Cases reported subsequently were characterized by ectropion of the lower eyelids, double row of eyelashes (distichiasis) of the upper eyelids, bilateral horizontal widening of the palpebral fissures (euryblepharon), bilateral CLP, and various features of ectodermal dysplasia, including dental manifestations ranging from oligodontia to agenesis and conical crown teeth [22, 23]. Elschnig syndrome was renamed BCDS in 1996 [21].

Transmission is autosomal dominant. *De novo* PV has also been reported [24, 25].

Other clinical features are imperforate anus, neural tube defect, hypothyroidism due to thyroid aplasia or hypoplasia, and syndactyly [24–29].

The clinical characteristics of BCDS patients, by gene involved, are detailed in Table 22.2.

22.3.2 Genes Associated with BCDS

In 2007, Freitas et al. were the first to look for a molecular basis to BCDS [33]. They studied genes associated with syndromes similar to BCDS, e.g., Hey–Wells syndrome (AEC) and ectrodactyly-ectodermal dysplasia-cleft syndrome EEC, in eight affected individuals. They did not observe any PV in *P63, IRF6, FOXE1, OSR2,* and *TBX10*.

A clue to the involvement of *CDH1* in this complex syndrome was first given in 2016 [32]. Nishi et al. performed WES in a girl with a syndromic presentation including CLP, meningoencephalocele, tetralogy of Fallot, and developmental delay, while her parents were unaffected. A missense variant of *CDH1* was identified in the girl but not in her parents, leading the authors to conclude that it was likely responsible for the phenotype.

In 2017, Ghoumid et al were the first to demonstrate convincingly that *CDH1* is associated with BCDS [24]. They first performed WES in five families with de novo BCDS. They found three missense variants in *CDH1* and two truncating variants in *CTNND1,* a gene coding for the catenin-delta 1, another adhesion protein. In a second stage, they did *CDH1* and *CTNND1* targeted sequencing in three additional affected families and identified one splice site variant and one in-frame deletion in *CDH1* and one nonsense variant in *CTNND1*. They confirmed the deleterious impact of *CDH1* variants by functional analysis. By RT-PCR on lymphocytes RNA, they confirmed *CDH1* exon 9 skipping due to the splice site variant, predicted to remove a major portion of the third extracellular domain implicated in adhesive function. One of the missense variants was predicted to also affect the splicing of exon 9 and the in-frame deletion was predicted to remove a conserved residue within the same extracellular domain. E-cadherin expression assay revealed no detectable protein

Table 22.2 Clinical characteristics of patients with blepharocheilodontic syndrome according to the gene involved

Features/study	Alharatani 2020 [30]	Leblanc 2020 [31]	Kievit 2018 [25]			Ghoumid 2017 [24]		Nishi 2016 [32]	Previous studies [24]	Total	
Causing gene	CTNND1	CDH1	CDH1	CTNND1	None	CDH1	CTNND1	CDH1	NA	CDH1	CTNND1
Number of carriers	15	4	18	8	–	8	5	1	NA	31	28
Number of families	9	1	12	3	2	5	3	1	NA	19	15
Inherited	4/9	1	4	2	–	2	2	0	NA	7	8
Asymptomatic carriers	1/13	0	0	0	–	1	1	0	NA	1	2
Female/Male gender	6/9	3/1	12/6	6/2	1/1	8/0	1/4	1/0	NA	24/7	13/15
Eyelid anomalies	13/14	3/4	16/17	7/8	2/2	7/8	4/5	1/1	29/35	27/30	24/27
Ectropion of lower eyelids	4/14	2/2	10/14	5/8	NA	7/8	3/5	0/1	29/35	19/25	12/27
Euryblepharon	NA	2/2	12/14	6/8	2/2	7/8	3/5	1/1	28/35	22/25	9/13
Lagophtalmia	NA	2/2	12/14	5/8	2/2	7/8	3/5	1/1	28/35	22/25	8/13
Distichiasis	4/14	NA	9/14	6/8	0/2	7/8	3/5	1/1	22/35	17/23	13/27
Hypertelorism	7/14	NA	11/17	6/8	2/2	7/8	4/5	1/1	NA	19/26	17/27
Narrow, upslanted palpebral fissures	9/14	NA	NA	NA	NA	NA	NA	1/1	NA	1/1	9/14
Lacrimal duct abnormalities	NA	NA	3/14	0/8	0/2	NA	NA	NA	NA	3/14	0/8
Ectodermal dysplasia	11/13	4/4	16/17	7/8	2/2	7/8	4/5	NA	26/35	27/29	22/26
Hair anomalies	NA	NA	9/16	3/8	1/2	6/8	3/5	NA	11/35	15/24	6/13
Conical teeth/ abnormal crown form	9/13	NA	12/14	5/8	1/2	5/8	4/5	NA	11/35	17/22	18/26
Tooth agenesis/ hypodontia	8/12	4/4	13/15	6/8	2/2	7/8	4/5	NA	26/35	24/27	18/25
Nail dysplasia	NA	NA	1/16	0/8	0/2	5/8	0/5	NA	3/31	6/24	0/13
Vertex aplasia	NA	NA	NA	NA	NA	2/8	0/5	NA	NA	2/8	0/5
Craniofacial	11/14	4/4	15/16	3/8	2/2	7/8	3/5	1/1	33/35	27/29	17/27
CLP	8/14	4/4	15/16	3/8	2/2	6/8	3/5	1/1	33/35	26/29	14/27
Choanal atresia	4/14	NA	0/12	0/8	NA	1/8	0/5	1/1		2/21	4/27

(continued)

Table 22.2 (continued)

Features/study	Alharatani 2020 [30]	Leblanc 2020 [31]	Kievit 2018 [25]			Ghoumid 2017 [24]		Nishi 2016 [32]	Previous studies [24]	Total	
Causing gene	CTNND1	CDH1	CDH1	CTNND1	None	CDH1	CTNND1	CDH1	NA	CDH1	CTNND1
Dysmorphism	13/14	3/4	16/17	8/8	2/2	7/8	4/5	1/1		27/30	25/27
Cardiac disease	6/14	1/4	NA	NA	NA	NA	NA	1/1		2/5	6/14
Tetralogy of Fallot	1/14	0/4	NA	NA	NA	NA	NA	1/1		1/5	1/14
Neurodevelopmental	8/14	NA	1/15	0/8	0/2	NA	NA	1/1		2/16	8/22
Neurodevelopmental delay	3/14	NA	1/15	0/8	0/2	NA	NA	1/1		2/16	3/22
Limb anomalies	9/14	NA	2/13	0/8	0/2	1/8	1/5	1/1	4/11	4/22	10/27
Syndactyly	4/14	NA	2/13	0/8	0/2	1/8	1/5	0/1	4/11	3/22	5/27
Cancer	1/14	2/4	0/16	0/8	NA	0/8	0/5	–		2/28	1/27
Gastric cancer	0/14	1/4	0/16	0/8	NA	0/8	0/5	–		1/28	0/27
Other anomalies	8/14	1/1	7/17	0/8	0/2	5/8	2/5	1/1		14/27	10/27
Congenital hypothyroidism	1/14	1/1	4/14	0/7	0/2	2/8	1/5	0/1	2/31	7/24	2/26
Anal atresia	NA	NA	2/16	0/8	0/2	2/8	0/5	0/1	3/31	4/25	0/13
Neural tube defects	NA	NA	3/15	0/8	0/2	2/8	0/5	1/1	1	6/24	0/13
Agenesis of corpus callosum	1/14	NA	NA	NA	NA	NA	NA	1/1		1/1	1/14
Other	Ovarian dysgerminoma (1/14)	Breast cancer and lung adenocarcinoma (1/4); branch pulmonary stenosis (1/4); epidermoid cyst (1/4); bilateral enlargement of vestibular aqueduct (1/4)	Hirsutism forehead (1/18), dermoid cyst (1/18), bilateral mixed loss (1/18)			Allodynia (2/8); gastric cancer in relative not tested		Lumbar vertebral fusion			

CLP cleft lip and palate

expression for three variants (two affecting the splice site of exon 9 and one missense variant).

Using WES in a cohort of 28 individuals from 17 different families with BCDS, Kievit et al. identified eight different *CDH1* variants; five missense variants coding for extracellular residues and three exon 9 splice site variants [25]. They showed that a splicing variant involving this donor splice site resulted in an in-frame deletion of 183 bp in *CDH1* mRNA causing a partial deletion of the third extracellular cadherin domain. They performed functional experiments in zebrafish by injecting mRNAs bearing *CDH1* variants in embryos and observed developmental defects affecting head structures. Palate defects were also described. On the contrary, expression of *CDH1* variants associated with HDGC in zebrafish did not show reduced effect on embryo development. They also tested the effect in human breast cancer cell line MCF7 in which *CDH1* was knocked out. By coexpressing WT and *CDH1* BCDS-related variants, they observed a colocalization of the WT and mutated forms and an impairment of cell-cell adhesion, whereas coexpression of HDGC variant was not colocalized with the WT form, but was rapidly internalized, suggesting a dominant negative effect of BCDS-related variants. Of note, the authors also reported three variants in *CTNND1*.

No DGC had been reported in BCDS families until 2020. Leblanc et al. described the case of a patient with *CDH1*-associated BCDS (variant c.768T>A/p.(Asn256Lys)) who was diagnosed with advanced DGC at the age of 37 [31]. Almost at the same time, Ghoumid et al. (2020) reported that one of their *CDH1*-BCDS case had a diagnosis of DGC following surveillance endoscopy [34]. Endoscopy had been offered because of a family history of gastric cancer, although with no confirmation of the histological type in the relative. The variant implicated was c.1320+1G>C.

A total of 31 carriers of *CDH1* variants in BCDS families are reported to date and 28 of *CTNND1* variants. The clinical characteristics of these patients are detailed in Table 22.2. Patients have similar features regardless of the gene involved, but patients with *CTNND1* variants are likely to have a less severe phenotype [24, 25, 30].

In the following section, we will summarize the function of E-Cadherin, the protein encoded by *CDH1* and explore the hypotheses that could explain the phenotype variability in *CDH1* PV carriers.

22.4 Physiopathology

22.4.1 Function of E-Cadherin

E-cadherin is involved in embryogenesis and maintenance of tissue architecture. It mediates cell-cell adhesion in a calcium-dependent manner. The mature form of E-cadherin has an ectodomain composed of five tandem repeats, a transmembrane domain, and a cytoplasmic domain. Cell adhesion function is mediated by the extracellular domain, which ensures proper folding and dimerization of

E-cadherins. The cytoplasmic domain interacts with p120, beta- and alpha-catenins anchored to the actin cytoskeleton [35].

During embryogenesis, E-cadherin is expressed since the 8-cell stage and allows the adhesion of blastomeres by polarization and differentiation, enabling the compaction of the morula and the subsequent organization of epithelia [36]. It plays an important role in craniofacial morphogenesis, especially during the formation of cartilage and facial bones, and the development of teeth. It plays its role either by controlling cell-cell adhesion or by intracellular Wnt signaling [37].

In adults, E-cadherin plays a key role in the maintenance and homeostasis of the epithelium. Notably, the reduction or absence of E-cadherin expression has been described in diffuse gastric and LBC. It disrupts epithelial morphology and increases tumor invasiveness through epithelial–mesenchymal transition [38].

Given these primary roles of E-cadherin, the phenotypes associated with *CDH1* variants are not surprising. We will now explore hypotheses that could account for their diversity.

22.4.2 Modifying Factors

It has been hypothesized that, apart from specific *CDH1* variants, environmental and/or other genetic factors may explain the incomplete penetrance. Environmental factors such as alcohol consumption or folate deficiency during pregnancy have been implicated. Maternal passive smoking in utero was reported for three carriers with CLP by Obermair et al. in 2019, whereas other unexposed carriers did not show CLP [13]. In particular, they described lower WT E-cadherin expression in a patient who was the only active smoker in the cohort supporting this hypothesis.

Differential levels of *CDH1* promoter methylation have also been shown between carriers with and without CLP in *CDH1* families [39]. In the case of HDGC, penetrance depends on the occurrence of a second hit, which is frequently a promoter hypermethylation on the non-mutated allele [40].

Oligogenic models, with modifier genes, could also influence the penetrance of HDGC and CLP. Genome-wide association studies suggest the existence of "modifying loci" containing potential regulatory regions [19].

22.5 Genotype–Phenotype Correlation

CDH1 PV associated with HDGC are mostly truncating variants distributed along the gene (nonsense variants, frameshift variants, or splicing variants leading to a premature stop codon). In families with a predisposition to cancer and CLP, there is no obvious specificity either in the type of variants or in their distribution within the gene. The same seems to apply to non-syndromic CLP without a family history of cancer. In contrast, in BCDS, the vast majority of *CDH1* variants are missense and splice site and are located in two mutational hotspots [24, 25].

To date, a total of 33 *CDH1* variants have been reported in non-syndromic and familial CLP (Table 22.1). Some have, however, now been classified as benign or probably benign, and many remain of unknown significance. Only two are truncating. There is a family history of DGC in eight cases, associated with splice site ($n = 6$) and truncating ($n = 2$) variants. Overall, if variants are distributed throughout the gene, the majority is located in extracellular domain regions (Fig. 22.1a).

Functional assays have been performed to assess whether non-syndromic CLP is associated to specific *CDH1* variants. For example, p.(Asp254Asn) and p.(Arg784His) induce cytoplasmic relocalization of E-Cadherin, with reduced expression for p.(Asp254Asn). Cells expressing both variants do not show invasive properties, possibly explaining the absence of cancer in these families [14]. However, five CLP variants have also been identified in HDGC families (p.(Pro30Thr); c.531+3A>G; c.532-18C>T; p.(Tyr341*); p.(Asp805Asn)) [14, 18]. Functional assays have been performed for p.(Pro30Thr) and p.(Asp805Asn), showing E-cadherin relocalization but no reduced expression or increased cell invasion [18]. It is possible that each variant behaves differently, inducing cell-specific biological behavior with distinct clinical impact [41].

As for BCDS, all 16 associated *CDH1* variants are in the extracellular domain of E-cadherin with apparent hotspots in the binding regions (Table 22.3). More precisely, variants are clustered around the 254–257 calcium-binding site between the two most distal extracellular (EC) domains, suggesting that disruption of calcium binding and thus interaction between cadherins could explain this particular phenotype. Furthermore, all variants associated with BCDS are missense variants or variants affecting the donor splice site at the exon 9- intron 9 junction and one in-frame deletion. The disruption of this splice site induces *CDH1* exon 9 skipping, predicted to remove a major portion of the third extracellular domain (Fig. 22.1b).

CDH1 variants associated with BCDS are therefore predicted to result in a mutant protein. The hypothesis of a dominant negative effect, first proposed by Frebourg et al. in 2006 [10] is strengthened by these observations. Kievit et al. did report a dominant negative effect in human cells and zebrafish associated with BCDS *CDH1* variants[25]. They observed that the mutant proteins dimerized with the wild-type ones, thus interfering with adhesion junctions.

22.6 Conclusion

Over the past 15 years, research has shown that the phenotypes associated with *CDH1* variants go far beyond cancer. The type of variants and their localization seems to differ between HDGC and CLP/BCDS, suggesting that different pathophysiological mechanisms would actually explain the different clinical manifestations. Clinical, molecular, and functional studies in larger CLP/BCDS cohorts are necessary in order to explore this likely genotype–phenotype correlation and provide much-needed data to clinical geneticists managing and counseling *CDH1* families.

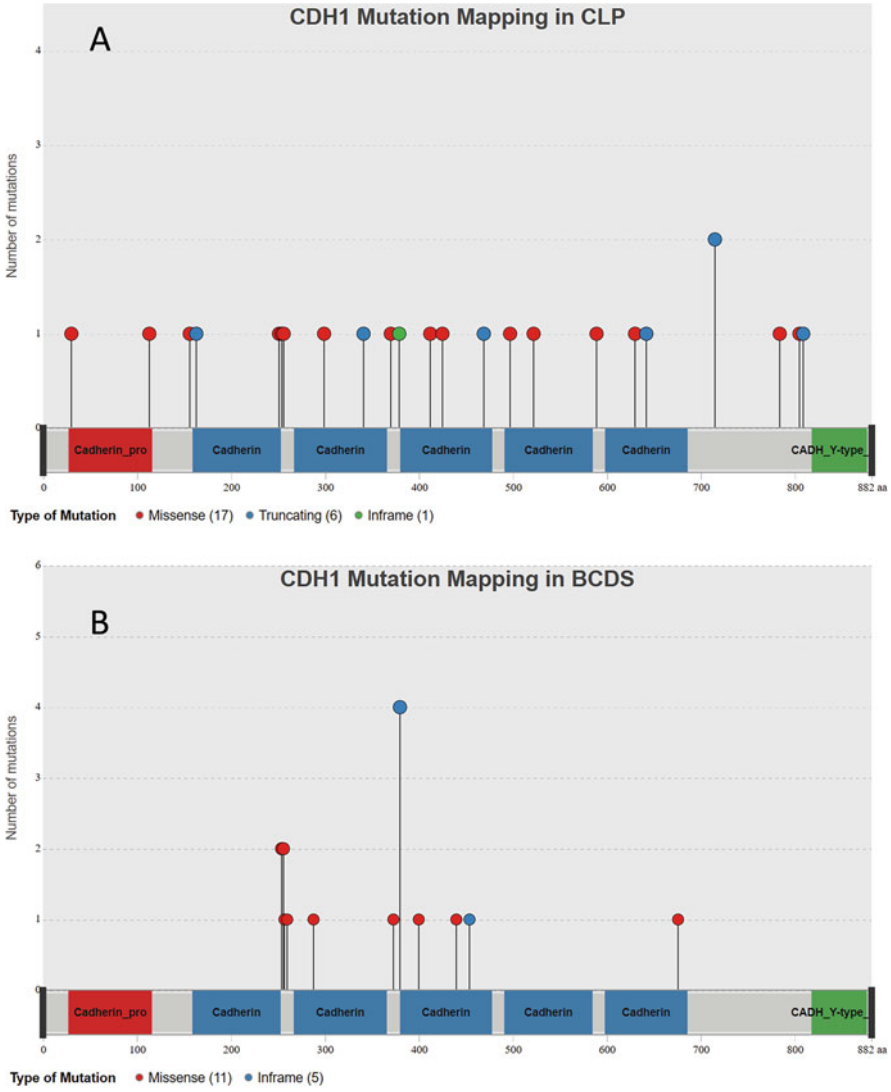


Fig. 22.1 (**a**) Lollipop plot of *CDH1* variants reported in Table 22.1 as associated to clefts of lip and palate, on the protein. (**b**) Lollipop plot of *CDH1* variants reported in Table 22.3 as associated to blepharocheilodontic syndrome, on the protein. The type of variants is indicated by colored circles (missense, truncating, or in-frame deletions). The height of lollipops corresponds to the number of individuals/families in who the variant has been identified. The four splice site variants (c.1320+1 or c.1320+5) inducing an in-frame deletion are noted as "in-frame" in this plot

Table 22.3 *CDH1* variants reported in patients with blepharocheilodontic syndrome

Domain	Mutation	Amino acid change	Location	Type	BCD cases	Cancer history	References	ClinVar	Selvanathan [19] (classification by ACMG criteria)
Extracellular	c.760G>A[a]	p.(Asp254Asn)	Exon 6	Missense	7		[25]	VUS*	P
	c.760G>T	p.(Asp254Tyr)	Exon 6	Missense	1		[24]	P	P
	c.768T>A	p.(Asn256Lys)	Exon 6	Missense	1+3	1 HDGC	[25, 31]: same family described by [15]	LP	LP
	c.768T>G	p.(Asn256Lys)	Exon 6	Missense	1		[25]	NA	-
	c.770A>T	p.(Asp257Val)	Exon 6	Missense	1		[24]	NA	P
	c.779C>T	p.(Pro260Leu)	Exon 6	Missense	1		[19]	NA	LP
	c.862G>C	p.(Asp288His)	Exon 7	Missense	1		[25]	NA	P
	c.1118C>G	p.(Pro373Arg)	Exon 8	Missense	3		[25]	VUS*	P
	c.1198G>A	p.(Asp400Asn)	Exon 9	Missense	1		[19]	VUS	LP
	c.1320G>T	p.(Lys440Asn)	Exon 9	Missense	2		[24]	P	P
	c.1320+1G> A	p.(Tyr380_Lys440del)	Intron 9	Splice site	3		[25]	NA	P
	c.1320+1G>C	p.(Tyr380_Lys440del)[b]	Intron 9	Splice site	2	GC (mother presumed to be carrier); 1 HDGC	[24, 34]	LP***	P
	c.1320+1G> T	p.(Tyr380_Lys440del)	Intron 9	Splice site	1		[25]	P/LP	P
	c.1320+5G> A	p.(Tyr380_Lys440del)	Intron 9	Splice site	1		[25]	NA	LP

(continued)

Table 22.3 (continued)

Domain	Mutation	Amino acid change	Location	Type	BCD cases	Cancer history	References	ClinVar	Selvanathan [19] (classification by ACMG criteria)
	c.1361_1369del	p.(Val454del)	Exon 10	In-frame deletion	1		[24]	NA	P
	c.2028C>A	p.(Asp676Glu)	Exon 13	Missense	1		[32]	VUS*	P

[a]Variant also identified in families with cleft lip palate only

[b]The amino acid change was not explicitly given by Ghoumid et al. but an exon 9 skipping was shown as for the other variants reported by Kievit et al. involving the donor splice site at the junction of exon 9-intron 9, the amino acid change was thus deducted from Kievit's study; ClinVar: review status:"*": criteria provided, single submitter, "**": criteria provided, multiple submitters, no conflicts, "***": reviewed by expert panel; otherwise: no assertion provided. [25]

GC gastric cancer, *HDGC* hereditary diffuse gastric cancer, *B* Benign, *LB* likely benign, *VUS* variant of unknown significance, *LP* likely pathogenic, *P* pathogenic

References

1. Dixon MJ, Marazita ML, Beaty TH, Murray JC (2011) Cleft lip and palate: understanding genetic and environmental influences. Nat Rev Genet 12:167–178. https://doi.org/10.1038/nrg2933
2. Tolarová MM, Cervenka J (1998) Classification and birth prevalence of orofacial clefts. Am J Med Genet 75:126–137
3. Watkins SE, Meyer RE, Strauss RP, Aylsworth AS (2014) Classification, epidemiology, and genetics of orofacial clefts. Clin Plast Surg 41:149–163. https://doi.org/10.1016/j.cps.2013.12.003
4. Sivertsen A, Wilcox AJ, Skjaerven R, Vindenes HA, Abyholm F, Harville E, Lie RT (2008) Familial risk of oral clefts by morphological type and severity: population based cohort study of first degree relatives. BMJ 336:432–434. https://doi.org/10.1136/bmj.39458.563611.AE
5. Butali A, Mossey PA, Adeyemo WL, Eshete MA, Gowans LJJ, Busch TD, Jain D, Yu W, Huan L, Laurie CA, Laurie CC, Nelson S, Li M, Sanchez-Lara PA, Magee WP, Magee KS, Auslander A, Brindopke F, Kay DM, Caggana M, Romitti PA, Mills JL, Audu R, Onwuamah C, Oseni GO, Owais A, James O, Olaitan PB, Aregbesola BS, Braimah RO, Oginni FO, Oladele AO, Bello SA, Rhodes J, Shiang R, Donkor P, Obiri-Yeboah S, Arthur FKN, Twumasi P, Agbenorku P, Plange-Rhule G, Oti AA, Ogunlewe OM, Oladega AA, Adekunle AA, Erinoso AO, Adamson OO, Elufowoju AA, Ayelomi OI, Hailu T, Hailu A, Demissie Y, Derebew M, Eliason S, Romero-Bustillous M, Lo C, Park J, Desai S, Mohammed M, Abate F, Abdur-Rahman LO, Anand D, Saadi I, Oladugba AV, Lachke SA, Amendt BA, Rotimi CN, Marazita ML, Cornell RA, Murray JC, Adeyemo AA (2019) Genomic analyses in African populations identify novel risk loci for cleft palate. Hum Mol Genet 28:1038–1051. https://doi.org/10.1093/hmg/ddy402
6. Ludwig KU, Mangold E, Herms S, Nowak S, Reutter H, Paul A, Becker J, Herberz R, AlChawa T, Nasser E, Böhmer AC, Mattheisen M, Alblas MA, Barth S, Kluck N, Lauster C, Braumann B, Reich RH, Hemprich A, Pötzsch S, Blaumeiser B, Daratsianos N, Kreusch T, Murray JC, Marazita ML, Ruczinski I, Scott AF, Beaty TH, Kramer F-J, Wienker TF, Steegers-Theunissen RP, Rubini M, Mossey PA, Hoffmann P, Lange C, Cichon S, Propping P, Knapp M, Nöthen MM (2012) Genome-wide meta-analyses of nonsyndromic cleft lip with or without cleft palate identify six new risk loci. Nat Genet 44:968–971. https://doi.org/10.1038/ng.2360
7. Yu Y, Zuo X, He M, Gao J, Fu Y, Qin C, Meng L, Wang W, Song Y, Cheng Y, Zhou F, Chen G, Zheng X, Wang X, Liang B, Zhu Z, Fu X, Sheng Y, Hao J, Liu Z, Yan H, Mangold E, Ruczinski I, Liu J, Marazita ML, Ludwig KU, Beaty TH, Zhang X, Sun L, Bian Z (2017) Genome-wide analyses of non-syndromic cleft lip with palate identify 14 novel loci and genetic heterogeneity. Nat Commun 8:14364. https://doi.org/10.1038/ncomms14364
8. Bureau A, Parker MM, Ruczinski I, Taub MA, Marazita ML, Murray JC, Mangold E, Noethen MM, Ludwig KU, Hetmanski JB, Bailey-Wilson JE, Cropp CD, Li Q, Szymczak S, Albacha-Hejazi H, Alqosayer K, Field LL, Wu-Chou Y-H, Doheny KF, Ling H, Scott AF, Beaty TH (2014) Whole exome sequencing of distant relatives in multiplex families implicates rare variants in candidate genes for oral clefts. Genetics 197:1039–1044. https://doi.org/10.1534/genetics.114.165225
9. Deshpande AS, Goudy SL (2019) Cellular and molecular mechanisms of cleft palate development. Laryngoscope Investig Otolaryngol 4:160–164. https://doi.org/10.1002/lio2.214
10. Frebourg T, Oliveira C, Hochain P, Karam R, Manouvrier S, Graziadio C, Vekemans M, Hartmann A, Baert-Desurmont S, Alexandre C, Lejeune Dumoulin S, Marroni C, Martin C, Castedo S, Lovett M, Winston J, Machado JC, Attié T, Jabs EW, Cai J, Pellerin P, Triboulet JP, Scotte M, Le Pessot F, Hedouin A, Carneiro F, Blayau M, Seruca R (2006) Cleft lip/palate and CDH1/E-cadherin mutations in families with hereditary diffuse gastric cancer. J Med Genet 43:138–142. https://doi.org/10.1136/jmg.2005.031385

11. Benusiglio PR, Caron O, Consolino E, Duvillard P, Coulet F, Blayau M, Malka D (2013) Cleft lip, cleft palate, hereditary diffuse gastric cancer and germline mutations in CDH1. Int J Cancer 132:2470–2470. https://doi.org/10.1002/ijc.27923
12. Kluijt I, Siemerink EJM, Ausems MGEM, van Os TAM, de Jong D, Simões-Correia J, van Krieken JH, Ligtenberg MJ, Figueiredo J, van Riel E, Sijmons RH, JTM P, van Hillegersberg R, Dekker E, Oliveira C, Cats A, Hoogerbrugge N, Cancer on behalf of the DWG on HG (2012) CDH1-related hereditary diffuse gastric cancer syndrome: clinical variations and implications for counseling. Int J Cancer 131:367–376. https://doi.org/10.1002/ijc.26398
13. Obermair F, Rammer M, Burghofer J, Malli T, Schossig A, Wimmer K, Kranewitter W, Mayrbaeurl B, Duba H-C, Webersinke G (2019) Cleft lip/palate and hereditary diffuse gastric cancer: report of a family harboring a CDH1 c.687 + 1G > A germline mutation and review of the literature. Familial Cancer 18:253–260. https://doi.org/10.1007/s10689-018-0111-5
14. Brito LA, Yamamoto GL, Melo S, Malcher C, Ferreira SG, Figueiredo J, Alvizi L, Kobayashi GS, Naslavsky MS, Alonso N, Felix TM, Zatz M, Seruca R, Passos-Bueno MR (2015) Rare variants in the epithelial cadherin gene underlying the genetic etiology of nonsyndromic cleft lip with or without cleft palate. Hum Mutat 36:1029–1033. https://doi.org/10.1002/humu.22827
15. Cox LL, Cox TC, Moreno Uribe LM, Zhu Y, Richter CT, Nidey N, Standley JM, Deng M, Blue E, Chong JX, Yang Y, Carstens RP, Anand D, Lachke SA, Smith JD, Dorschner MO, Bedell B, Kirk E, Hing AV, Venselaar H, Valencia-Ramirez LC, Bamshad MJ, Glass IA, Cooper JA, Haan E, Nickerson DA, van Bokhoven H, Zhou H, Krahn KN, Buckley MF, Murray JC, Lidral AC, Roscioli T (2018) Mutations in the epithelial cadherin-p120-catenin complex cause mendelian non-syndromic cleft lip with or without cleft palate. Am J Hum Genet 102:1143–1157. https://doi.org/10.1016/j.ajhg.2018.04.009
16. Du S, Yang Y, Yi P, Luo J, Liu T, Chen R, Liu C-J, Ma T, Li Y, Wang C, Weng J, Liu M, Zhang L, Yang B, Zeng X, Liu JY (2019) A novel CDH1 mutation causing reduced E-cadherin dimerization is associated with nonsyndromic cleft lip with or without cleft palate. Genet Test Mol Biomarkers 23:759–765. https://doi.org/10.1089/gtmb.2019.0092
17. Ittiwut R, Ittiwut C, Siriwan P, Chichareon V, Suphapeetiporn K, Shotelersuk V (2016) Variants of the CDH1 (E-Cadherin) gene associated with oral clefts in the Thai population. Genet Test Mol Biomarkers 20:406–409. https://doi.org/10.1089/gtmb.2015.0325
18. Vogelaar IP, Figueiredo J, van Rooij IALM, Simões-Correia J, van der Post RS, Melo S, Seruca R, Carels CEL, Ligtenberg MJL, Hoogerbrugge N (2013) Identification of germline mutations in the cancer predisposing gene CDH1 in patients with orofacial clefts. Hum Mol Genet 22:919–926. https://doi.org/10.1093/hmg/dds497
19. Selvanathan A, Nixon CY, Zhu Y, Scietti L, Forneris F, Uribe LMM, Lidral AC, Jezewski PA, Mulliken JB, Murray JC, Buckley MF, Cox TC, Roscioli T (2020) CDH1 mutation distribution and type suggests genetic differences between the etiology of orofacial clefting and gastric cancer. Genes (Basel) 11:E391. https://doi.org/10.3390/genes11040391
20. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17:405–424. https://doi.org/10.1038/gim.2015.30
21. Gorlin RJ, Zellweger H, Curtis MW, Wiedemann H-R, Warburg M, Majewski F, Gillessen-Kaesbach G, Pranl-Anderson B, Zackai E (1996) Blepharo-cheilo-dontic (BCD) syndrome. Am J Med Genet 65:109–112. https://doi.org/10.1002/(SICI)1096-8628(19961016)65:2<109::AID-AJMG5>3.0.CO;2-N
22. Allanson JE, McGillivray BC (1985) Familial clefting syndrome with ectropion and dental anomaly—without limb anomalies. Clin Genet 27:426–429. https://doi.org/10.1111/j.1399-0004.1985.tb02288.x
23. Korula S, Wilson L, Salomonson J (1995) Distinct craniofacial syndrome of lagophthalmia and bilateral cleft lip and palate. Am J Med Genet 59:229–233. https://doi.org/10.1002/ajmg.1320590221

24. Ghoumid J, Stichelbout M, Jourdain A-S, Frenois F, Lejeune-Dumoulin S, Alex-Cordier M-P, Lebrun M, Guerreschi P, Duquennoy-Martinot V, Vinchon M, Ferri J, Jung M, Vicaire S, Vanlerberghe C, Escande F, Petit F, Manouvrier-Hanu S (2017) Blepharocheilodontic syndrome is a CDH1 pathway-related disorder due to mutations in CDH1 and CTNND1. Genet Med 19:1013–1021. https://doi.org/10.1038/gim.2017.11
25. Kievit A, Tessadori F, Douben H, Jordens I, Maurice M, Hoogeboom J, Hennekam R, Nampoothiri S, Kayserili H, Castori M, Whiteford M, Motter C, Melver C, Cunningham M, Hing A, Kokitsu-Nakata NM, Vendramini-Pittoli S, Richieri-Costa A, Baas AF, Breugem CC, Duran K, Massink M, Derksen PWB, van IJcken WFJ, van Unen L, Santos-Simarro F, Lapunzina P, Gil-da Silva Lopes VL, Lustosa-Mendes E, Krall M, Slavotinek A, Martinez-Glez V, Bakkers J, van Gassen KLI, de Klein A, van den Boogaard M-JH, van Haaften G (2018) Variants in members of the cadherin-catenin complex, CDH1 and CTNND1, cause blepharocheilodontic syndrome. Eur J Hum Genet 26:210–219. https://doi.org/10.1038/s41431-017-0010-5
26. Ababneh FK, Al-Swaid A, Elhag A, Youssef T, Alsaif S (2014) Blepharo-cheilo-dontic (BCD) syndrome: expanding the phenotype, case report and review of literature. Am J Med Genet A 164A:1525–1529. https://doi.org/10.1002/ajmg.a.36465
27. da Silva G, Lopes VL, Guion-Almeida ML, de Oliveira Rodini ES (2003) Blepharocheilodontic (BCD) syndrome: expanding the phenotype? Am J Med Genet A 121A:266–270. https://doi.org/10.1002/ajmg.a.20223
28. Martinhago CD, Ramos ES (2004) Blepharo-cheilo-dontic (BCD) syndrome with agenesis of thyroid broadens the phenotype and extends the discussion about the genes. Am J Med Genet A 130A:437–438. https://doi.org/10.1002/ajmg.a.30285
29. Weaver KN, Rutledge KD, Grant JH, Robin NH (2010) Imperforate anus is a rare associated finding in blepharocheilodontic syndrome. Am J Med Genet A 152A:438–440. https://doi.org/10.1002/ajmg.a.33207
30. Alharatani R, Ververi A, Beleza-Meireles A, Ji W, Mis E, Patterson QT, Griffin JN, Bhujel N, Chang CA, Dixit A, Konstantino M, Healy C, Hannan S, Neo N, Cash A, Li D, Bhoj E, Zackai EH, Cleaver R, Baralle D, McEntagart M, Newbury-Ecob R, Scott R, Hurst JA, Au PYB, Hosey MT, Khokha M, Marciano DK, Lakhani SA, Liu KJ (2020) Novel truncating mutations in CTNND1 cause a dominant craniofacial and cardiac syndrome. Hum Mol Genet 29:1900–1921. https://doi.org/10.1093/hmg/ddaa050
31. LeBlanc S, Naveen D, Haan E, Barnett C, Rawlings L, Roscioli T, Poplawski N (2020) CDH1-related blepharocheilodontic syndrome is associated with diffuse gastric cancer risk. Am J Med Genet A 182:1780–1784. https://doi.org/10.1002/ajmg.a.61601
32. Nishi E, Masuda K, Arakawa M, Kawame H, Kosho T, Kitahara M, Kubota N, Hidaka E, Katoh Y, Shirahige K, Izumi K (2016) Exome sequencing-based identification of mutations in non-syndromic genes among individuals with apparently syndromic features. Am J Med Genet A 170:2889–2894. https://doi.org/10.1002/ajmg.a.37826
33. Freitas ÉL, Martinhago CD, Ramos ES, Murray JC, Gil-da-Silva-Lopes VL (2007) Preliminary molecular studies on blepharocheilodontic syndrome. Am J Med Genet A 143A:2757–2759. https://doi.org/10.1002/ajmg.a.32010
34. Ghoumid J, Lejeune S, Renaud F, Stichelbout M, Petit F, Manouvrier-Hanu S (2020) Confirmation of risk of cancer in blepharocheilodontic syndrome. Genet Med 22:1727–1728. https://doi.org/10.1038/s41436-020-0849-7
35. Takeichi M (1995) Morphogenetic roles of classic cadherins. Curr Opin Cell Biol 7:619–627. https://doi.org/10.1016/0955-0674(95)80102-2
36. Fleming TP, Javed Q, Hay M (1992) Epithelial differentiation and intercellular junction formation in the mouse early embryo. Dev Suppl:105–112
37. Schambony A, Kunz M, Gradl D (2004) Cross-regulation of Wnt signaling and cell adhesion. Differentiation 72:307–318. https://doi.org/10.1111/j.1432-0436.2004.07207002.x

38. van Roy F, Berx G (2008) The cell-cell adhesion molecule E-cadherin. Cell Mol Life Sci 65: 3756–3788. https://doi.org/10.1007/s00018-008-8281-1
39. Alvizi L, Ke X, Brito LA, Seselgyte R, Moore GE, Stanier P, Passos-Bueno MR (2017) Differential methylation is associated with non-syndromic cleft lip and palate and contributes to penetrance effects. Sci Rep 7:2441. https://doi.org/10.1038/s41598-017-02721-0
40. Oliveira C, Sousa S, Pinheiro H, Karam R, Bordeira-Carriço R, Senz J, Kaurah P, Carvalho J, Pereira R, Gusmão L, Wen X, Cipriano MA, Yokota J, Carneiro F, Huntsman D, Seruca R (2009) Quantification of epigenetic and genetic 2nd hits in CDH1 during hereditary diffuse gastric cancer syndrome progression. Gastroenterology 136:2137–2148. https://doi.org/10.1053/j.gastro.2009.02.065
41. Figueiredo J, Söderberg O, Simões-Correia J, Grannas K, Suriano G, Seruca R (2013) The importance of E-cadherin binding partners to evaluate the pathogenicity of E-cadherin missense mutations associated to HDGC. Eur J Hum Genet 21:301–309. https://doi.org/10.1038/ejhg.2012.159

Hereditary Breast Cancer Non-*CDH1* Associated

23

Rosa Di Micco, Emanuela Esposito, Giuseppe Accardo, Andrea Sibilio, Andriana Kouloura, Mara Costa, and Oreste Davide Gentilini

Abstract

Although breast cancer (BC) mainly arises as a sporadic tumor, between 7 and 10% of BC patients present a pathogenic variant (PV) of selected genes. This prevalence was calculated on high-risk populations and could be even higher in general population. Women with BC susceptibility or hereditary BC represent a special setting of patients who deserve a personalized approach to their screening and treatment pathways. PVs of BRCA1 and BRCA2 genes are implicated in 15% of all familial BCs. Additionally, increasing evidence is available on germline mutations in several other genes which are associated to inherited susceptibility to BC. These PVs have been stratified according to their penetrance, although guidelines are somewhat discordant on this classification. PVs in high-penetrance genes, i.e., BRCA, p53, PTEN, STK11, PALB2, CDH1, carry a threefold or more increased risk of BC compared to the general population,

R. Di Micco (✉) · O. D. Gentilini
Breast Surgery Unit, IRCCS San Raffaele Research and University Hospital, Milan, Italy
e-mail: dimicco.rosa@hsr.it

E. Esposito
Department of Breast Cancer, Istituto Nazionale Tumori, IRCCS Fondazione Giovanni Pascale, Naples, Italy

G. Accardo
Department of Breast Surgery, Centro di Riferimento Oncologico della Basilicata (IRCCS-CROB), Rionero in Vulture, Potenza, Italy

A. Sibilio
Breast Surgery Unit (Forlì-Ravenna), AUSL Romagna, Ravenna, Italy

A. Kouloura
Department of Breast Surgery, Athens Euroclinic Hospital, Athens, Greece

M. Costa
Breast Surgery Unit, IRCCS Multimedica, Sesto San Giovanni, Milan, Italy

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_23

moderate-penetrance genes, i.e., CHEK2, BARD1, ATM, lead to a twofold to threefold risk, whilst low-penetrance genes, i.e., RAD51, BRIP1, NF1, are associated to a onefold to twofold risk of BC. The deep knowledge of hereditary BC-associated genes and the identification of PVs is crucial to provide the proper prevention strategy in carriers and a targeted therapy in BC patients.

23.1 Introduction

The idea of hereditary breast cancer (BC) was born in the late nineteenth century when Paul Broca first described his wife's high-risk familial pedigree. However, the theory of inherited susceptibility to BC started developing in the 1980s with the research carried on by the geneticist Mary-Claire King, who was able to identify a BC-associated gene on chromosome 17q21 [1]; this gene was soon after named *breast cancer susceptibility gene 1* (*BRCA1*).

Even though most BCs are sporadic, 15% of patients present a *familial* BC with one or more first/second-degree relatives with the disease or a *hereditary* BC harboring a genetic predisposition to develop cancer. In fact, between 7.8 and 10.2% of women with a BC carry a pathogenic variant (PV), i.e., a deleterious germline mutation of selected genes which increases BC risk [2, 3]. These mutations mostly affect tumor suppressor genes, which are involved in DNA damage recognition and repair pathways. In this scenario, high-penetrance genes *BRCA1* and *BRCA2*, play a leading role and their PVs are shown in about 15% of all women with familial BC [4]. When considering well-defined, high-risk Hereditary Breast and Ovarian Cancer syndromes (HBOC), this percentage rises to 52% [5]. *BRCA1/2* germline mutations are found in 3–4% of all women with BC, including 10–20% of those with triple-negative BC (TNBC) and 10–15% of affected women with Jewish ancestry [6] (Table 23.1).

To date, increasing evidence is available on several germline mutations associated with inherited susceptibility to BC, which are distinct according to their penetrance. BC-associated deleterious gene mutations have been stratified into groups of high penetrance (carrying a threefold or more increased risk of BC compared to the general population), moderate penetrance (twofold to threefold risk), or low penetrance (onefold to twofold risk). When considering high-penetrance genes the most relevant are protein *p53* (*TP53*) and *phosphatase and tensin homolog tumor suppressor* (*PTEN*) genes, determining Li–Fraumeni (LFS) and Cowden syndrome (CS), respectively; *STK11* gene causing the Peutz-Jeghers syndrome (PJS), and *CDH1* gene which will be treated in other chapters. Additionally, moderate penetrance genes are *partner and localizer of BRCA2* (*PALB2*) gene (which is also considered highly penetrant gene in some guidelines), *checkpoint kinase 2* (*CHEK2*), and *ataxia-telangiectasia mutated* (*ATM*) genes (at low penetrance in some guidelines), which account for a smaller percentage of BCs [7]. Couch et al. reported that, in a population of 41,611 women with BC, most commonly mutated non-*BRCA1/2* genes among white women were *CHEK2*

Table 23.1 BRCA pathogenetic/likely pathogenetic variant-positive management

Gene	Absolute breast cancer risk	Management	
		Surveillance	Risk Reduction Mastectomy (RRM)
BRCA1	>60%	• Clinical breast exam every 6–12 months starting at age 25 years • Breast screening – Age 25–29 years: annual breast MRI with contrast – Age 30–75 years: annual breast MRI with contrast and annual mammogram with consideration of tomosynthesis – Age > 75 years: individualized management	• Discuss option of RRM
BRCA2	>60%		

Adapted from National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic, Version 1.2022

(1.73%), *ATM* (1.06%), and *PALB2* (0.87%) [4]. Globally, mutations of moderate penetrance genes are encountered in 4–6% of BC patients [6].

Due to improvements in expertise and reduction of costs of genetic testing, the list of genes associated with BC susceptibility is continuously evolving but in most cases definitive evidence on the associated cancer risk is still lacking, above all when variants of unknown significance (VUS) are diagnosed. In a recent cohort study including more than 200,000 patients with breast or ovarian cancer who were diagnosed between 2013 and 2017 and tested with Multi-Gene Panels, VUS-only rates markedly increased during time from 8.1 to 28.3%, thus outpacing the increase in PVs and highlighting racial or ethnic differences in VUS-only rates related to under-representation of non-Caucasian ethnicities in clinical trials [8]. Moreover, classification of mutations penetrance among different international societies may result divergent. ATM, for instance, is considered a moderate penetrance gene by American Society of Clinical Oncology (ASCO) guidelines and a low-penetrance gene by St. Gallen panelists, while PALB2 is considered highly penetrant by St. Gallen International Consensus and intermediate by ASCO [6, 9]. However, although the consequences on clinical practice of an increase in VUS diagnosis rate or of those classification differences are unknown. We can assume that it might bring to patients' overtreatment or excess of risk-reducing procedures.

Afterall, the knowledge of these issues together with the identification of mutation carriers is crucial, since it may warrant a potential change in care or prevention strategy for patients. Nevertheless, the selection of women who should undergo genetic testing is still highly debated, and recommendations are not univocal. On one side, the American Society of Breast Surgeons has recommended genetic testing for

all BC patients, on the other side the U.S. Preventive Services Task Force and National Comprehensive Cancer Network (NCCN) suggested a selection of healthy women too [10–12]. As regards the management of carriers, the 2021 St. Gallen International Consensus favored the consideration of risk-reducing mastectomy for women harboring PVs in highly penetrant genes (e.g., BRCA1, BRCA2, TP53, and PALB2), and surveillance with mammography and magnetic resonance imaging (MRI) for women with PVs in moderate penetrance genes (e.g., BARD1, CHEK2, CDH1, and STK11). For women with less penetrant gene pathogenic mutations (such as ATM, BRIP1, NF1, RAD51C, and RAD51D), surveillance was prompted without prophylactic mastectomy [9]. Differences in terms of prevalence between mutations of BRCA1/2 compared to other genes have led to a much deeper knowledge of BC genetics. This translated into very strong evidence-based guidelines for surveillance strategies and management of pathogenetic BRCA mutation carriers, developing not only specific risk-reduction screening and surgical plans but also different local management strategies and targeted therapies for affected patients [13] (Tables 23.1 and 23.2). In contrast, the management of other less common mutation carriers still needs to be better defined (Tables 23.2 and 23.3).

23.2 Genetics of BC

Research on genes associated with cancer inheritance is one of the main focuses of modern literature on BC. The genetic variations found in female BC fall into two distinct categories. The first one is the gain-of-function mutations in the *proto-oncogenes* which induce the cell to grow and to divide. The other one is the loss-of-function mutations in *tumor suppressor genes* which result in uncontrollable cell growth, inability to repair DNA damage and lack of cell cycle check points. Women who inherit loss-of-function mutations have a 70% chance of developing BC by the time they are 70 years old. Cancer predisposition genes are often distinguished according to their penetrance, i.e. the estimate that a specific condition like cancer will occur in the presence of a specific genotype. By the way, there is still no consensus about the classification of genes according to their penetrance: Table 23.3 displays the difference between classification according to diverse guidelines, resulting in different therapeutic and prevention strategies [6, 12, 14].

23.2.1 High-Penetrance Genes

Among highly penetrant mutated genes, *BRCA1* and *BRCA2* genes represent 16% of all genes associated to hereditary BC. Most of these genes are involved in multiple hereditary syndromes that are associated with the highest lifetime risks for BC such as HBOC for BRCA1–2, LFS for TP53, the PTEN hamartoma syndrome (PHTS) for PTEN, the hereditary diffuse gastric cancer (HDGC) for CDH1, and PJS for STK11. These syndromes are rare and they only represent approximately 5% of non-sporadic BCs. They are inherited in an autosomal dominant pattern and typically have

Table 23.2 Breast cancer risk and management of high- and moderate-penetrance genes with pathogenic mutations

Gene	Absolute breast cancer risk Surveillance	Management Risk reduction mastectomy (RRM)	
TP53	>60%	• Breast screening – Age 20–29 years: annual breast MRI with contrast – Age 30–75 years: annual breast MRI with contrast and annual mammogram with consideration of tomosynthesis	Discuss option of RRM taking into consideration family history
PTEN	40–60%	• Clinical breast exam every 6–12 months starting at age 25 years or 5–10 years before the earliest known BC in the family • Breast screening; annual mammogram with consideration of tomosynthesis and consider MRI with contrast starting at age 35 years or 10 years before the earliest known BC in the family	Discuss option of RRM taking into consideration family history
PALB2	41–60%	Annual mammogram with consideration of tomosynthesis and consider MRI with contrast starting at age 30 years	Discuss option of RRM
ATM	15–40%	Annual mammogram with consideration of tomosynthesis and consider MRI with contrast starting at age 40 years	Insufficient evidence, manage based on family history
BARD1	Evidence limited, but stronger for TNBC	Annual mammogram with consideration of tomosynthesis and consider MRI with contrast starting at age 40 years	Insufficient evidence, manage based on family history
CHEK2	15–40%	Annual mammogram with consideration of tomosynthesis and consider MRI with contrast starting at age 40 years	Insufficient evidence, manage based on family history
STK11	40–60%	• Clinical breast exam every 6 months starting at age 30 years • Breast screening; annual mammogram and MRI with contrast starting at age 30 years	Insufficient evidence, manage based on family history
NF1	15–40%	Annual mammogram with consideration of tomosynthesis starting at age 30 years and consider MRI with contrast from age 30–50 years	Insufficient evidence, manage based on family history

Adapted from NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic. Vers.1, 2022

Table 23.3 Grade of penetrance of genes associated to BC susceptibility according to different guidelines

	Gene mutation penetrance			
Guidelines	ASCO	St. Gallen	NCCN	IGCLC
BRCA1	High	High	High	–
BRCA2	High	High	High	–
PTEN	High	–	High	–
PALB2	Moderate	High	High	–
BARD1	–	Moderate	Moderate	–
CHEK2	Moderate	Moderate	Moderate	–
CDH1	High	Moderate	High	High
STK11	High	Moderate	Moderate	–
TP53	High	Moderate	High	–
ATM	Moderate	Low	Moderate	–
RAD51D	–	Low	Moderate	–
RAD51C	–	Low	Moderate	–
BRIP1	–	Low	Moderate	–
NF1	–	Low	Moderate	–
NBN	–	Low	Moderate	–
FANCC	–	Low	–	–

ASCO American Society of Clinical Oncology, *IGCLC* International Gastric Cancer Linkage Consortium

younger than average ages of cancer diagnosis. There is risk for more than one tumor type, not only for BC (Table 23.4), and there is a specific pattern of early onset cancers in successive generations of the affected family. Genetic testing has a key role in the detection of the causative mutation of hereditary predisposition to cancer in these families, thus addressing PV carriers to more intensive screening and prevention options than general population [15].

23.2.1.1 BRCA1–BRCA2: Hereditary Breast and Ovarian Cancer Syndrome

The most common hereditary cause of BC is HBOC, which is caused by a pathogenic germline mutation in either the BRCA1 or BRCA2 gene. It follows an autosomal dominant pattern and the occurrence of malignancies in germline mutation carriers encompasses somatic inactivation of the remaining allele of the involved gene. Estimates of lifetime BC risk in women vary from 41 to 80%. Recent estimates from metanalysis and prospective analysis are 57% and 60% for BRCA1 mutation carriers and 49% and 55% for BRCA2 mutation carriers, respectively. Additionally, the risk to age 70 years for a contralateral BC (CBC) is estimated to be 50% or higher. As regards male carriers, their risk of BC is 5–10% for BRCA2 mutation carriers and 1–2% for BRCA1 mutation carriers. In addition to BC, women with a BRCA1 or BRCA2 mutation have a particularly increased risk for epithelial ovarian (40–59% for BRCA1, 16.5–18% for BRCA2), fallopian tube, and primary peritoneal cancers (see Table 23.4) [16].

Table 23.4 Syndromes associated to BC susceptibility genes, most frequent mutations, and types of cancer with increased risk

Syndrome	Gene or locus (chromosomal location)	Type of cancer
Hereditary breast/ovarian cancer syndrome	BRCA1 (17q12–21) BRCA2 (13q12–13)	BC, ovarian cancer BC, ovarian, prostate and pancreatic cancer, melanoma
Li–Fraumeni syndrome	TP53 (17p13.1)	BC, sarcomas, leukemia, brain tumors, adrenocortical and lung carcinoma
Cowden syndrome	PTEN (10q23.3)	BC, thyroid, endometrial cancer. Other: benign hamartomas, macrocephaly
Peutz-Jeghers syndrome	STK11 (19p13.3)	BC, ovarian, cervical, uterine, testicular, small bowel and colon cancer. Other: Hamartomatous polyps of the small intestine, mucocutaneous pigmentation
Hereditary gastric cancer	CDH1 (16q22.1)	Hereditary diffuse gastric, lobular BC, colorectal cancer
Lynch syndrome	MLH1, MSH2, MSH6, PMS2 (3P22.2; 2P21–P16, 2P16.3, 7P22.1)	BC, ovarian, endometrial, stomach and colorectal cancer
Ataxia telangiectasia	ATM (11q22.3)	BC, ovarian, prostate and pancreatic, stomach, bladder and colon cancer
CHEK2 related	CHEK2 (22q12.1)	BC, colorectal, ovarian, bladder, prostate, thyroid, serous uterine, gastric and kidney cancer
PALB2 related	PALB2 (16p12.1)	BC, pancreatic, ovarian cancer, male BCs. Other: Fanconi Anemia
	BARD1 (2q34–q35), BRIP1 (17q22–q24), MRE11A (11q21), NBS1 (8q21), RAD50 (5q31), RAD51C (17q25.1), XRCC2 (7q36.1), RAD51D (17q11), ABRAXAS (4q21.23)	BARD1: BC and ovarian cancers MRE11: ataxia telangectasia-like disorder RAD50: Nijmegen breakage syndrome like disorder NBS1: Nijmegen breakage syndrome

BRCA proteins are multifunctional and control homologous recombination (HR), DNA repair mechanism (DSB, double-strand breaks), and cellular response to DNA damage. Germline mutations that define HBOC are responsible for the reduced capacity of the cell to repair DNA DSBs and high-level chromosomal instability. The repair occurs by HR through replication, utilizing the homologous strand as a template [17]. BRCA1 protein also acts as a transcriptional activator or repressor with a key role in chromatin remodeling and in the regulation of centrosomes. In detail, BRCA1 and BRCA2 function as tumor suppressor genes and their mutations have been identified throughout their coding sequences and in proximity to the

splicing sites. A total of 1639 different mutations and polymorphism in BRCA1 genes have been reported by the BC Information Core (BIC) 2010 database. In addition, 1853 unique mutations, polymorphisms, and variants in the BRCA2 genes were also reported. These mutations could be frameshifts, non-sense or splice-site mutations and deletions. They result in a shortened BRCA1/2 protein which fails to perform its physiologic function [18]. It is estimated that 1 in 300–800 individuals carry a deleterious germline mutation in one of these genes, though mutations are more common in certain populations due to *founder mutations.* For instance, approximately 1 in 40 individuals of Ashkenazi Jewish ancestry carries one of the three specific founder mutations, (187delAG or 5385insC in BRCA1 or 6174delT in BRCA2) [19].

Imaging BRCA mutation carriers should undergo close monitoring, although the sensitivity of standard imaging could be different when compared with general population. In particular, mammography showed a lower sensitivity (around 37%, range: 19–50%) in these patients. This effect is mainly due to the masking effect of dense breast tissue in young women, to the potentially benign appearance of BRCA-associated BC and the low incidence of ductal carcinoma in situ and associated microcalcifications in BRCA1 carriers. BRCA1-related BC generally shows benign morphologic features like a smooth non-spiculated mass, with round or oval shape and circumscribed margins. Additionally, due to the rapid tumor growth, the rates of false negative results and interval cancer are high. Similarly, ultrasound screening for high-risk women showed low sensitivity (17–52%), no universal recommendations exist for BRCA carriers. BRCA-related tumors appear like hypoechoic masses with an irregular shape, parallel orientation, and non-circumscribed margins. On the contrary, contrast-enhanced MRI is the most sensitive imaging tool in this population, showing a sensitivity of 71–100% and a specificity of 79–98%. Although guidelines recommend MRI in high-risk patients, data on mortality reduction with MRI are poor. However, a downward shift of BC stage and a reduction in interval cancer rates in patients screened with MRI could be eventually considered as a surrogate endpoint that correlates with a reduction in mortality. MRI findings in BRCA-related tumors are of outstanding importance for differential diagnosis with benign disease. In detail, the presence of rim enhancement together with the enhancement kinetics, the lack of internal septations which are typical in fibroadenomas, asymmetric non-mass enhancement with focal, regional or segmental distribution are additional essential diagnostic data. Despite variable screening schedule proposed, the annual screening with MRI from 25 years and the additional mammography starting from the age of 30 years resulted as the most cost-effective regimen [12, 20, 21].

Surgery Risk reduction surgeries for BRCA mutation carriers have been widely spread over the last two decades. Prophylactic bilateral mastectomy and prophylactic oophorectomy are both surgical strategies being used for BC prevention among BRCA carriers, although results have not been assessed by randomized controlled trials in high-risk women.

Bilateral risk-reducing mastectomy (BRRM) has been shown to be the most effective approach to reduce BC risk in women diagnosed with BRCA mutation. Population-based studies have demonstrated carriers having elevated rate of ipsilateral recurrence and CBC [22]. There is evidence that BRRM decreases the incidence of BC by 90–95% in women who have a deleterious mutation in the BRCA1 and/or BRCA2 gene, although benefits in survival are still unclear [23–27]. Nipple sparing mastectomy (NSM) is considered the preferred approach for risk reducing surgery among BRCA mutation carriers as current surgical techniques have improved cosmetic outcomes. However, as NSMs have been associated with residual glandular breast tissue, the oncological safety in terms of risk reduction in this subset of women is of particular concern for some authors [28]. In order to prevent occult BC, pre-surgical mammography and magnetic resonance imaging (MRI) should be performed no later than 6 months prior to the surgery [29]. The prophylactic purpose of the BRRM highlights the importance of a natural aesthetic outcome, which can be achieved by different immediate autologous and implant-based breast reconstruction techniques (either through pre-pectoral or sub-pectoral approach). Therefore, risk-reducing surgery should be performed by a surgical team with specialist skills in oncoplastic surgery and breast reconstruction in high-volume breast centers [30–32].

A preoperative detailed discussion of advantages and drawbacks for mutation carriers considering risk-reducing mastectomy is mandatory. Topics should include the likely prognosis of their BC; their chance of developing a distal recurrence of their previous BC; a clear quantification of the risk of developing BC in the other breast; the potential negative impact of mastectomy on body image and sexuality; the different appearance and feel of the breasts after reconstructive surgery (https://www.nice.org.uk/guidance/cg164/chapter/recommendations). For women with newly diagnosed BC who have a deleterious mutation in BRCA1 and/or BRCA2 gene, NSM is a reasonable oncologic approach to consider with low rate of locoregional recurrence and low complication rates [6]. In this setting, breast conserving surgery (BCS) could also be offered to women eligible for, although the strength of this recommendation is moderate and it is essential to counsel patients regarding the elevated risk of ipsilateral second primary BC and CBC. Literature reports the 15-year risk of ipsilateral BC recurrence to be fourfold higher among carriers compared to non-carriers (23 vs. 6.4%) and this risk increases with time [33]. However, no significant differences in overall survival and BC specific survival have been observed among carriers between BCS and mastectomy [34]. Radiation therapy can be offered to BRCA 1 and/or BRCA2 carriers with BC for whom it is indicated, because there is no evidence of increased toxicity or CBC events from radiation exposure in this subset of patients [6]. For women with BC who have a BRCA1 and/or BRCA2 mutation and who have been treated or are being treated with unilateral mastectomy, contralateral risk reducing mastectomy (CRRM) should be offered because CRRM is associated with a decreased risk of CBC although there is insufficient evidence for improved survival. Carriers have an annual CBC rate of approximately 2–3% compared to 0.5% in patients with sporadic BC [35, 36]. The risk of CBC is greater among women whose initial BC occurs at a young age than in older counterparts and among individuals with a BRCA1 PV when compared to

BRCA2 mutation carrier [37–39]. That said the following factors should be considered for assessing risk of CBC and the role of risk-reducing mastectomy in BRCA1 and/or BRCA2 mutation carriers: age at diagnosis, family history of BC, compliance of patient to undergo breast surveillance with MRI, comorbidities, and life expectancy.

Most patients diagnosed with BC are not aware of having a BRCA mutation prior to surgery. Patients with preoperative awareness of BRCA mutation more often opt for BRRM. Chiba et al. underlined the impact that the timing of genetic mutation diagnosis on the surgical decision-making process and showed that 59% of patients who underwent primary BCS decided for delayed BRRM after knowing the result of the test [40]. Patients at high risk of mutation should be tested before initial surgery to make an informed decision together with their surgeon on which therapeutic strategy to approach after specific genetic counseling [41]. The NCCN guidelines recommend to test affected or unaffected individuals who have a probability >5% of a *BRCA1/2* PV based on probability models (e.g., BRCAPro, BOADICEA) [12]. The National Institute for Care and Health Institute (NICE) guidelines in UK recommend offering genetic testing to people with a 10% (or bigger) likelihood of carrying a *BRCA1/2* mutation (https://www.nice.org.uk/guidance/cg164/chapter/recommendations). As regards men carrying BRCA mutation, annual clinical breast exam from the age of 35 years is recommended, whereas risk-reducing surgery is not advised [12].

Pathology BC arising in BRCA carriers usually exhibits distinct histopathological characteristics. Around 80% of BRCA-associated BC are invasive ductal carcinomas of no special type that are poorly differentiated and highly proliferative [42]. In particular, grade 3 tumors account for 66–84% of cases vs. 30–40% in sporadic age-matched BC. Furthermore, immunohistochemical subtypes are different in BRCA1- and BRCA2-associated BC. BRCA1-related tumors are mainly triple negative (TNBC) and show more often medullary features (13 vs. 2% in non-carriers) together with prominent lymphocyte infiltrate in the surrounding microenvironment, foci of necrosis, pushing margins and high frequency of lympho-vascular invasion [43] Furthermore, they exhibit less tubule formation, higher pleomorphism, and more mitosis than do sporadic controls. As regards hormone receptors, in BRCA1 carriers less than 25% of BCs express estrogen (ER) or progesterone (PgR) receptors and only 10% exhibit Her2 gene amplification, whilst the majority of BRCA2-associated tumors are ER- and PgR- positive with percentages of 65 and 40–60%, respectively [44]. BRCA2 tumors are characterized by pushing margins and lack of tubule formation, being similar to sporadic luminal BC with no specific morphological phenotype [43, 45]. Within hereditary BC, there is a subset of carcinomas called "basal-like." This phenotype is characterized by ER- and Her2-negativity and the expression of specific "basal cell" or myoepithelial markers, such as high-molecular weight cytokeratin (CK) CK5/6, CK14 and CK17, and P-Cadherin. In addition, basal-like BC shows overexpression of cyclin E and downregulation of p27, with higher expression of p53, neuroendocrine markers (chromogranin A and synaptophysin), stem-cell-phenotype markers (CD44+/

CD24−), and others (i.e., hypoxia-associated factor; CA9; FHIT protein) that are absent in triple negative non-basal like cancer [45]. Moreover, the overexpression of epidermal growth factor receptor (EGFR) is also considered part of the special features of the basal phenotype [46]. Additionally, EGFR mutations are present in 45% of BRCA-related tumors compared to 15% of sporadic BC. Similarly, p53 protein-truncating mutations are present in 100% of BRCA1 tumors and are strongly correlated to basal-like phenotype and have been also described in 29–63% of BRCA2 tumors, thus confirming that p53 may promote tumorigenesis in BRCA-deficient tumors [45]. Results from the POSH study reported that 7% of BRCA1-associated and 11% of BRCA2-associated tumors were Her2-positive [47].

Systemic Therapy Hereditary BC represents a unique entity that requires tailored strategies in terms of targeted systemic therapies in addition to standard therapy of sporadic BC. In fact, BRCA 1/2 deficiency, down-regulation of DNA DSB repair, and significant chromosomal instability characterize BRCA-associated tumors and form the basis for their susceptibility pattern to unique treatment opportunities such as cytotoxic drugs, DNA-damaging agents, poly (ADP-ribose) polymerase (PARP) inhibitors, and ionizing radiation [48].

- *Platinum salts*: Platinum-based cytotoxic drugs deploy their cytotoxic effects by crosslinking and damaging DNA strands. They form DNA crosslinks that cause damaged DNA DSBs activating DNA repair by HR, which is unavailable in BRCA-mutated patients. Therefore, cells with HR deficiency are susceptible to platinum salts [49]. Platinum compounds, especially cisplatin, are currently used in the neoadjuvant, adjuvant, and also in the metastatic setting of hereditary BC. Byrski et al. found a pCR rate of 61% in a cohort of 107 BRCA1 mutation carriers with stage I–III BC treated with four cycles of neoadjuvant cisplatin [50]. Additionally, the phase II CALBG 40603 trial on patients with stage II and III TNBC the pCR rate was higher with carboplatin added to neoadjuvant chemotherapy. However, there was no improvement in the long-term survival outcome [51]. A recent meta-analysis by Chai et al. aimed at assessing the values of BRCA status and HR deficiency in the prediction of the pCR rated of patients with TNBC undergoing platinum-based neoadjuvant regimen. They concluded that pCR rates were significantly higher in HR deficiency-positive patients than those HR deficiency negative (241/412, 58.5% vs. 60/183, 32.8%, OR, 3.01; 95% CI, 2.07–4.39, $p < 0.001$), thus proving that BRCA1/2 mutated patients with deficiency in HR and TNBC could benefit from platinum-based neoadjuvant chemotherapy [49]. The Triple-negative BC trial (TNT) was the largest trial assessing the role of platinum drugs in the treatment of BRCA 1/2 mutation carriers with metastatic TNBC. The overall response rates in the BRCA 1/2 mutation carriers' group were higher for the group that received carboplatin vs. docetaxel (68 vs. 33%) [52].
- *Taxanes*: The mechanism of action of antimicrotubule agents is blocking cell division, and specifically taxanes disrupt the microtubule function, which is essential to this process. Several studies have evaluated whether the BRCA status

could impact on toxicity and outcomes of taxane-based treatment. Boughey et al. evaluated the response to neoadjuvant weekly paclitaxel followed by adriamycin/cyclofosfamide (FEC) in patients with stage I–III BC, including 12 BRCA mutated patients. They reported no association between BRCA status and response rate to taxanes, additionally most MRI responses were observed in the BRCA2 group [53]. The phase II GeparSixto study showed that the efficacy of taxane in combination with anthracycline is higher in BC. Additionally, the pCR increased by 25% with carboplatin for BRCA mutation carriers [54]. In general, the clinical benefit of standard chemotherapy regimen with antracycline and taxanes is confirmed in BRCA carriers, and specific data on taxane-related toxicity are very poor. However, according to Bayraktar et al. BRCA germline deleterious mutation does not increase the risk for peripheral neuropathy or hematological toxicity despite the ineffective DNA repair mechanism, whilst BRCA2 carriers may have a major risk of chemo-related gastrointestinal toxicity [55].

- *PARP Inhibitors*: The inhibitors of the enzyme poly-ADP ribose polymerase (PARP) lead to the trapping of PARP proteins on DNA and block their catalytic action causing cell death, especially in cancer cells that grow faster than the other non-cancerous cells. PARP1 is a crucial protein in repairing single-strand DNA breaks, and if strand breaks persist unrepaired until DNA replication, the replication itself can cause DSBs. PARP inhibitors cause multiple DSBs, and consequently, in tumors with BRCA1, BRCA2, and PALB2 mutations, these breaks cannot be repaired by HR, leading to cell death [56]. These compounds include *olaparib*, *rucaparib*, *niraparip*, and *talazoparib*, which act by trapping PARPs to DNA and *veliparib* that withholds the catalytic activity of PARP. Two phase III trials, OlympiAD and EMBRACA, showed improved progression-free survival with two PARP inhibitor monotherapies, *olaparib* and *talazoparib* compared with standard chemotherapy [57, 58]. These pharmacological compounds have been initially approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for locally advanced and metastatic HER2-negative BC in germline BRCA mutated patients. More recently, results from the OlympiA trial showed that in high-risk patients with Her2-negative BC and germline BRCA1/2 pathogenic or likely PVs adjuvant *olaparib* after local treatment and neoadjuvant or adjuvant chemotherapy was associated with significantly longer invasive disease-free survival (85.9 vs. 77.1% in the placebo group, 99.5% CI, 0.41 to 0.82; $p < 0.001$) and distant disease-free survival (87.5 vs. 80.4% in the placebo group, 99.5% CI, 0.39–0.83; $p < 0.001$) at 3-year follow-up [59]. Furthermore, in the BROCADE3 trial, *veliparib* has shown promising outcomes with regard to BRCA mutated locally advanced/metastatic BC in combination with platinum-based chemotherapy [60]. To date, PARP inhibitor therapies are under evaluation in novel combinations in the early stages of BC, including patients without germinal BRCA mutations and somatic BRCA mutations or mutations in other genes [61, 62].
- *Immunotherapy*: The immune system's role in limiting cancer progression is object of multiple clinical trials. The genetic alterations produce tumor-specific

neoantigens that provoke a T-cell response inside the tumor microenvironment. The microenvironment regarding BC releases immune suppressive factors that harm the immune response but mostly make the antigen presentation difficult. BC has not been conventionally considered immunogenic since immune infiltrates' mutational load and extent are lower than other tumor types. Both BRCA1 and BRCA2 carriers present tumors with high amounts of tumor-infiltrating lymphocytes (TILs) indicating that they are highly immunogenic BC and offer new perspectives to immune modulation therapy by checkpoint inhibitors and to chemotherapy which facilitates the "immunogenic cell death." In general, increasing levels of stromal TILs (sTILs) have been associated to improved rates of recurrence and death in TNBC and Her2-positive BC (for each 10% increase of sTILs, 8 and 10% reduction of mortality and disease-free survival event was observed), whilst the impact on prognosis in ER-positive BC translates into better prognosis in high-grade BC and worse prognosis in low- and intermediate-grade BC [63]. Monoclonal antibodies that block checkpoint receptors such as cytotoxic T lymphocyte-associated antigen (CTLA4) and programmed death-1 and its ligand (PD-1 and PD-L1) can stimulate an endogenous antitumor immune response [64]. Data suggest that combined treatment with chemotherapy and other immunotherapy agents, especially for TNBC cancer, can provide an effective antitumor immune response. Several clinical trials are currently underway to evaluate the efficacy of checkpoint inhibitors either as monotherapy or as an adjunct to PARP inhibitors or chemotherapy [65, 66].

23.2.1.2 TP53: Li–Fraumeni Syndrome

LFS was first described in 1969 and is an autosomal dominant predisposition to soft tissue sarcomas and osteosarcomas. Patients are characterized by an impressive pattern of early onset and multiple primary cancers such as BC, brain tumors, adrenocortical tumors, and many other malignancies (Table 23.4) [67]. Patients with LFS are particularly susceptible to radiation-induced malignancies, which complicates risk estimation for second primary tumors [68]. Overall cancer risk is estimated to be 50% by age 30 years and 90% by age 60, and is higher in women than men, primarily due to BC risk. Approximately, the 51% of females affected has a BC with median age of onset of 34 years [69]. Furthermore, there is emerging evidence of anticipation in LFS families (i.e., cancers occurring at progressively younger ages in subsequent generations of a family) or that genetic modifiers may affect penetrance [70]. LFS accounts for approximately 1% of BC cases overall, though risks for germline TP53 mutations are higher in women with early onset (younger than age 35) BC [34, 35]. Thus, early onset BC is now being included in the NCCN criteria for offering TP53 testing, even if there is no other family history of cancer [12, 71].

TP53 is a tumor suppressor gene which encodes for a protein responsible of various stress signals and suppression of cellular transformation via mediating cell-cycle, the cellular response against oncogenic stress, cell repair, and apoptosis. The most part of mutations of the gene is located in exons 5–8, spanning the

DNA-binding domain of the protein. The single-nucleotide germline missense mutation at exon 10 codon 337 of TP53 (CGC to CAC) has been described as the responsible for a change in arginine to histidine (R337H) that is associated to early onset of BC. On the other hand, overexpression of missense mutant TP53 was identified in different types of cancers and the tumor-promoting Gain-Of-Function activities have also been described, indicating its potential for targeting therapy [72].

Surgery Guidelines recommend against RT in women with BC who are carriers of a TP53 mutation because of the risk of radiation induced sarcoma [12, 14]. Thus, TP53 mutation carriers diagnosed with BC must be treated with mastectomy with or without reconstruction, and the option of risk-reducing mastectomy should be discussed amongst healthy women who carry TP53 mutation [12].

Pathology BC in LFS appears to be predominantly invasive ductal carcinoma enriched for HER2-positive status, and 84% are either ER- and/or PgR- positive. Additionally, malignant phyllodes tumor is strongly associated with LFS [73].

Systemic Therapy Studies on mice models showed that radiotherapy and genotoxic chemotherapies significantly increase the risk of multiple primary malignancies. Therefore, in TP53 mutation carriers with BC the surgical treatment should be prioritized followed by non-genotoxic chemotherapy, whilst radiotherapy must be avoided [74].

23.2.1.3 PTEN: Cowden Syndrome/PTEN Hamartoma Syndrome

Germline PTEN mutations have been identified in a variety of disorders with overlapping clinical features, including CS, Bannayan–Riley–Ruvalcaba syndrome, and Proteus-like syndrome, Lhermitte–Duclos disease, and autism with macrocephaly. Clinical diagnostic criteria for these conditions have evolved over time and to date PHTS is essentially preferred as a more specific term to describe individuals with germline PTEN mutations. The typical lesions of PHTS are *hamartomas*, which are benign tumors resulting from overgrowth of normal tissue, preferably affecting the skin, mucous membranes, breast, thyroid, endometrium, and brain [75–77] Other clinical findings that are strongly associated and highly predictive of PHTS are described in Table 23.4.

PTEN is a tumor suppressor gene with various functions such as regulation of cell cycle, apoptosis, and metastasis. Mutations or inactivation of PTEN gene were detected in 30% of BCs and can lead to hyper-activation of the PI3K/Akt signaling pathway. Germline mutations in PTEN are the cause of PHTS. The loss of PTEN function plays a key role in tumorigenesis and contribute to the resistance to cancer therapy. As mutations in PTEN are commonly de novo, a negative family history should not dissuade from the decision to offer PTEN genetic testing. Benign breast lesions are found in 76% of CS patients, while BC is the most commonly observed malignant tumor (30–50%) in CS, often discovered at a younger age (38–46 years) than average. Lifetime cancer risk for BC in women with PHTS ranges from 25 to 85% [78].

Surgery The option of BRRM should be discussed with PTEN mutation carriers. Counseling should include reconstructive options and possible complications. Psycho-social aspects and quality of life after prophylactic surgery should be highlighted. Moreover, the option of risk reducing hysterectomy upon completion of childbearing should be offered [12].

Systemic Therapy There is no direct evidence that the use of tamoxifen or raloxifene in these patients reduces the risk of developing BC. So, chemoprevention should be considered individually, taking into account the already increased risk of endometrial cancer in this group of patients [79].

Inhibitors targeting the PI3/AKT/mTOR pathway are considered a promising treatment for malignancies in individuals with a germline PTEN PV. There are limited specific treatment options for patients with CS. A phase II open-label clinical trial (NCT00971789) showed improvement of symptoms utilizing the mTOR inhibitor *sirolimus* that could inhibit cancer cell growth by blocking mTOR protein [80].

23.2.1.4 PALB2

PALB2 (also known as FANCN given that biallelic mutations cause a subset of Fanconi anemia) is involved in HR and DSB repair along with BRCA2. The PALB2 and BRCA2 proteins are tumor suppressors involved into the regulation of the cell growth and division. PALB2 mutations have primarily been associated with pancreatic cancer and BC (including bilateral female BC and male BC), and weak association with ovarian cancer susceptibility. Relative risk for BC is estimated to be 2.3-fold, perhaps higher, and may be mutation dependent [81]. The Finnish founder mutation ca.1592delT has an estimated 40% risk of BC to age 70 years and has been associated with higher incidence of TNBC and may have a negative impact on survival [82]. Around 10 mutations in PALB2 gene have been identified in BC patients. These mutations occur in a single copy of gene in each cell and result in an abnormally short version of PALB2 protein, which cannot interact effectively with BRCA2 protein to repair the damaged DNA [81, 83].

Surgery Recommendations and guidelines on PALB2 mutation carrier management are not unanimous. NCCN and St. Gallen Consensus Panel allocate PALB2 amongst high-penetrance genes and suggest discussing the option of BRRM, whilst ASCO accounts PALB2 amongst moderate-penetrance genes, thus recommending surveillance over risk-reducing surgery [12, 14].

Pathology PALB2 mutations were mostly associated to high-grade ductal carcinomas with ER-, PgR-, and Her2-negativity. PALB2 tumors were mainly CK5/6, CK14, and CK17 negative, showed high expression of Ki67 and low expression of Cyclin D1 as compared with other familial and sporadic patients [83, 84].

Systemic Therapy Apart from standard chemotherapy, PALB2 mutation carriers could also benefit from more tailored therapy. The phase II EBCRC 048 trial

explored the effectiveness of *olaparib* in metastatic BC patients with somatic or germline mutation in HR-related genes other than BRCA1/2. PARP inhibition with *olaparib* resulted to be effective in patients with germline mutation in PALB2 with a median progression free survival of 13.3 months [85].

23.2.1.5 STK11: Peutz-Jeghers Syndrome

PJS is a rare autosomal dominant condition characterized by mucocutaneous pigmentations, multiple hamartomatous gastrointestinal polyps, and increased risk of multiple types of cancer. Individuals have a cumulative lifetime risk of malignancy between 47 and 85%. The highest risks for malignancy are seen in the breast and colon, and the lifetime risk for BC in females ranges from 30 to 50% with an average onset of >30 years [86]. Liver Kinase B protein (LBK1), encoded by the STK11 tumor suppressor gene, is involved in a complex required for the activation of AMP-activated protein Kinase (AMPK) that is an energy metabolic sensor and is involved in cell polarity regulation and mediation of apoptosis. Recent studies have showed that mutations on threonine kinase gene STK11 (on 19p) play a crucial role in developing PJS. It is well known that LBK1 may only achieve mutations in individuals affected with PJS. Mutations of a single allele in LBK1 may be responsible for an aggressive BC with less survival chance [87]. Approximately 45% of affected individuals do not have any family history suggestive of PJS, indicating a high rate of de novo STK11 mutations. Clinical diagnostic criteria for PJS have been established, and when met, mutation detection rate in the STK11 gene is up to 94% [88].

Surgery STK11 is classified as a high-penetrance gene by ASCO guidelines but moderate by St. Gallen International Consensus. Evidence is still insufficient to suggest BRRM, but this could be considered according to family history. Therefore, surveillance with annual MRI is advised over surgery for healthy carriers. BCS along with radiotherapy should be offered to carriers diagnosed with BC [12, 14].

Systemic Therapy Although mTOR inhibitors have been used in PJS patients, there is limited data to suggest the efficacy of mTOR-targeted treatment in PJS patients with BC (Clinicaltrials.gov NCT00811590. Cinicaltrials.gov. NCT01178151).

23.2.2 Moderate-Penetrance Genes

Despite the increasing evidence about highly penetrant syndromes, most familial BC cases are not associated to mutations in the above-mentioned genes. Rather, a large proportion of these cancers may be due to combined effects from multiple relatively common low- or moderate-penetrance susceptibility genes as well as through environmental and lifestyle risk factors [89]. The hunt for additional BC susceptibility genes has led to the discovery of a class of relatively rare “moderately penetrant” genes and a class of relatively common “low penetrant” genes, which have been

found through candidate gene approaches as well as genome wide association studies (GWAS). For women with newly diagnosed BC who have a mutation in a moderate-penetrance BC gene, mutation status alone should not determine surgical decisions for the BC surgery or CRRM [6].

23.2.2.1 CHEK2

CHEK2 is a signaling protein involved in DNA repair. In particular, it plays a key role in the regulation of p53 function and BRCA1. Several types of mutations have been found, but the most studied is the *1100delC*, a common mutation in individuals of the Northern and Eastern European ancestry. This particular mutation leads to an abnormal, short and non-functional version of CHEK2 protein and it has consistently been found to be associated with higher BC risk in multiple studies. The prevalence of *1100delC* is moderately high in young onset BC cases (3.7% among women diagnosed younger than age 50 years) and familial BC cases (2.1–3.1%). An increased risk for CBC has been seen in many but not all studies, and there appears to be a more complex association with the *1100delC* mutation and bilateral disease [90, 91]. On the other hand, *CHEK2p.I157T* variant is linked to lower BC risk (about 1.5) [92]. Moreover, mutations including *S428F and CHEK2del5567* have been described in other populations, where *CHEK2del5567* seems to increase the risk of female BC by about twofold [93]. Lifetime BC risk is estimated at 20–44% but appears to be impacted by family history of BC. Women being homozygous for this mutation have significantly higher BC risk compared to heterozygotes [94]. CHEK2-associated tumors usually showed ER-positivity, a *U157T* mutation was associated to lobular carcinomas [18, 84].

Surgery BCS along with RT can be offered to women with the CHEK2 missense mutation *I157T*, whilst mastectomy should be discussed among those with women with the CHEK2 frameshift mutation *1100delC*. The risk of CBC is also variant specific and data are still scant, therefore evidence is insufficient to recommend BRRM amongst those women [92, 95].

Systemic Therapy Systemic therapy should be administered following the same recommendation available for non-carriers and may include chemotherapy, endocrine therapy, and targeted therapy according to molecular subtype.

23.2.2.2 ATM (Ataxia Telangectasia Mutated Gene)

ATM gene encodes for a protein kinase that is involved in activating cellular responses to DNA DSB via phosphorylation of key factors in the DNA damage-response pathway. Individuals having a deletion in one ATM gene copy in each cell are at high risk of BC. As a matter of the fact, cells missing half of the normal amount of ATM protein result in occurrence of mutations in other genes. Women harboring the ATM *c.7271T>G* as a pathogenic mutation may present higher BC risk and the penetrance seems to be same as that associated with germline BRCA2 mutations, although limited evidence is now available [96, 97]. ATM gene biallelic mutations are causative of ataxia telangiectasia, a childhood onset progressive neurological

disorder associated with telangiectasias, immunodeficiency, sensitivity to ionizing radiation, and increased risks for malignancies. The risk of BC is estimated at 15–52%, and the relative increase in risk may be even higher for women younger than age 50 years [98]. ATM-related tumors frequently show ER-positivity [84].

Surgery ATM carriers may need different strategies for treatment or surveillance, as the presence of a heterozygous mutation may increase susceptibility to morbidity from radiation and from certain chemotherapeutic agents. Furthermore, radiation exposure from mammography may increase BC risk in carriers, but data are still scant. There are insufficient data to recommend BRRM for ATM carriers. Annual surveillance is recommended over surgery, BCS along with RT should be offered when clinically indicated [6, 12, 14]. Data regarding rates of RT toxicity between ATM mutation carriers and non-carriers are limited and inconsistent [34, 99].

23.2.2.3 BARD1

BARD1 (BRCA Associated Ring Domain 1) interacts with BRCA1 in DSB repair and with p53 to promote apoptosis initiation or to regulate cell division. Biallelic BARD1 mutations also cause a subset of Fanconi anemia, and BARD1 suspected deleterious mutations have been found in 2.8% of BRCA1/BRCA2 negative breast/ovarian cancer families and 3.6% of high-risk BC families [100] with a frequent association to ER-negative BC [84]. In a previous study, missense *BARD1 variant Cys557Ser* has been revealed to be highly upregulated in BC families [101].

Surgery There are insufficient data to recommend risk-reducing mastectomy among BARD1 mutation carriers although there is a potential increased risk of TNBC. Therefore, surveillance with annual MRI is advised over surgery for healthy carriers. BCS along with radiotherapy should be offered to carriers diagnosed with BC [12, 14].

23.2.3 Low-Penetrance Genes

GWAS have been conducted for several years in order to identify common *polymorphisms* more likely to be involved in BC cases rather than in controls. Such studies assess the association of a large number of polymorphisms, which are common in the general population (found in at least 1%), with a particular disease. The polymorphism in such cases is not necessarily causative of a particular disease but may be physically close to a genetic region that might be. Of the single nucleotide polymorphisms (SNPs) identified by GWAS to be associated with BC, most have only a modest effect (OR < 1.5) [102]. The candidate gene approach has also identified several low-penetrance susceptibility genes for BC, noting that BC penetrance estimates are still to be defined [71].

23.2.3.1 RAD51 and RAD51 Related Genes (Including RAD51C, RAD51D, and XRCC2)

The family of RAD51 genes is involved in DNA damage repair via the HR pathway and interacts with BRCA1/BRCA2, PALB2 and p53. Dysregulation of RAD51 is capable of impairing HR and inducing aberrant genome rearrangements, genetic phenomena that can be seen in various cancers [103]. Several studies have assessed the association between a common *RAD51 polymorphism c.135G>C* and effect on BC risk among female BRCA1/BRCA2 mutation carriers, as well as in women whose BRCA1/BRCA2 status is unknown [104].

Biallelic mutations in *RAD51C* cause a Fanconi anemia-like condition. The literature on RAD51C provides evidence on an association with ovarian cancer and weak association with BC. Heterozygous mutations have been found in a small subset (1.3%) of familial breast and ovarian cancer families as well as unselected cases of ovarian cancer, but to date, no mutations have been identified in BC only families [105]. Similarly, *RAD51D* deleterious mutations have been found in a small subset (0.9%) of breast/ovarian cancer families, but not in BC only families, and the relative risk for BC was not significantly increased [106]. RAD51C and RAD51D mutation carriers have an absolute BC risk overtime spanning from 15 to 40%, especially for triple negative or ER-negative subtypes [84]. Recent data form BC Association Consortium placed protein-truncating variant in RAD51C and RAD51D in the moderate risk category and this shed a new light on these genes, suggesting a better tailored screening and prevention pattern for carriers [84].

XRCC2 was initially identified by an exome sequencing study in two BC families. Deleterious or predicted deleterious mutations were found in a higher percentage of population-based BC cases than controls and in approximately 1.5% of multiple case BC families and in no control cases [107].

Surgery BRRM is not recommended among healthy carriers. For carriers with newly diagnosed BC, surgical management is based on familial history. BCS followed by RT should be the standard approach until data on ipsilateral and contralateral BC rates will have been clarified. Additionally, the option of RRSO from 40 to 45 years should be considered [6, 12, 14].

23.2.3.2 BRIP1 (BRCA1 Interacting Protein C-Terminal Helicase 1)

BRIP1 germline mutation is associated with increased risk of breast and ovarian cancers. BRIP1 mutation confers high to moderate risk of ovarian cancer, thus RRSO from 40 to 45 years should be considered [12]. The impact of BRIP1 mutation on BC remains controversial, although most newly diagnosed BCs among these women are triple negative subtypes. We still have insufficient evidence for BC management. Surveillance over BRRM is now recommended and BCT along with RT is now indicated among patients who are suitable for [6, 12, 14].

23.2.3.3 NF1 (Neurofibromatosis Type 1)

NF1 mutation carriers have an increased risk of BC spanning from 15 to 40%. Available data strongly support the hypothesis that certain constitutional mutation

types with specific variants in NF1 confer different risks of BC. Risk for young onset BC (before age of 50 years) has also been reported to be increased four to fivefold among women with neurofibromatosis disease type 1 [108, 109]. However, there are insufficient data to recommend BRRM to NF1 germline mutation carriers. For carriers with newly diagnosed BC, BCS along with RT should be recommended [12, 14].

23.2.3.4 MRN Complex (Including MRE11, RAD50, and NBS1)

Meiotic Recombination 11 (MRE11), RAD50, and Nijmegen Breakage Syndrome 1 (NBS1) encode proteins that form an important complex repairing DSB and telomere maintenance, as well as DNA replication and cell cycle checkpoints [110]. Biallelic mutations in these genes cause an ataxia telangiectasia-like disorder and Nijmegen breakage type syndromes, respectively. Heterozygous mutations in these three genes have been detected with low frequencies (1–2%) in a Finnish population studied for having breast and ovarian cancer susceptibility. Among three genes in the MRN complex, the inherited NBS1 gene alteration has the strongest evidence to act as an intermediate-risk BC gene. A specific mutation in *NBS1 (657del5)* has been found to have a significant association with BC risk in a recent metanalysis. The BC risk associated with this mutation ranges from two to threefold [111].

Increasing attention is focusing on *ABRAXAS* gene (also known as ABRA1, CCDC98, or FAM175A). Mutations have recently been found in high-risk BC families and it is proposed to be a rare novel BC susceptibility gene [15].

BC has been reported in families with hereditary colon cancer predisposition syndromes including *Lynch syndrome and MUTYH-associated polyposis*, but a consistent causal link has not been demonstrated yet [84, 112–114].

23.3 Conclusions

The introduction of genetic testing in the management of BC has paved the way to a new era of precision medicine based on individual cancer risk. However, recommendations about the best way to screen or treat women with a genetic predisposition to BC are still heterogeneous.

To date, the knowledge of the different mutations and their clinical implications, the evaluation of other risk factors like familial history, patient's age, and individual preference, including self-risk perception and comfort with the various approaches, should definitely be the key drivers of any clinical choice.

References

1. Hall MJ, Bernhisel R, Hughes E, Larson K, Rosenthal ET, Singh NA et al (2021) Germline pathogenic variants in the ataxia telangiectasia mutated (ATM) gene are associated with high and moderate risks for multiple cancers. Cancer Prev Res 14(4):433–440

2. Couch FJ, Shimelis H, Hu C, Hart SN, Polley EC, Na J et al (2017) Associations between cancer predisposition testing panel genes and breast cancer. JAMA Oncol 3(9):1190–1196
3. Kurian AW, Ward KC, Howlader N, Deapen D, Hamilton AS, Mariotto A et al (2019) Genetic testing and results in a population-based cohort of breast cancer patients and ovarian cancer patients. J Clin Oncol 37(15):1305–1315
4. Couch FJ, Nathanson KL, Offit K (2014) Two decades after BRCA: setting paradigms in personalized cancer care and prevention. Science 343(6178):1466–1470
5. Kapoor NS, Curcio LD, Blakemore CA, Bremner AK, McFarland RE, West JG et al (2015) Multigene panel testing detects equal rates of pathogenic BRCA1/2 mutations and has a higher diagnostic yield compared to limited BRCA1/2 analysis alone in patients at risk for hereditary breast cancer. Ann Surg Oncol 22(10):3282–3288
6. Tung NM, Boughey JC, Pierce LJ, Robson ME, Bedrosian I, Dietz JR et al (2020) Management of hereditary breast cancer: American Society of Clinical Oncology, American Society for Radiation Oncology, and Society of Surgical Oncology Guideline. J Clin Oncol 38(18): 2080–2106
7. Hu C, Hart SN, Gnanaolivu R, Huang H, Lee KY, Na J et al (2021) A population-based study of genes previously implicated in breast cancer. N Engl J Med 384(5):440–451
8. Kurian AW, Ward KC, Abrahamse P, Bondarenko I, Hamilton AS, Deapen D et al (2021) Time Trends in Receipt of Germline Genetic Testing and Results for Women Diagnosed With Breast Cancer or Ovarian Cancer, 2012–2019. J Clin Oncol 39(15):1631–1640
9. Burstein HJ, Curigliano G, Thurlimann B, Weber WP, Poortmans P, Regan MM et al (2021) Customizing local and systemic therapies for women with early breast cancer: the St. Gallen International Consensus Guidelines for treatment of early breast cancer 2021. Ann Oncol 32(10):1216–1235
10. Owens DK, Davidson KW, Krist AH, Barry MJ, Cabana M, Caughey AB et al (2019) Risk assessment, genetic counseling, and genetic testing for BRCA-related cancer: US preventive services task force recommendation statement. JAMA 322(7):652–665
11. King MC, Levy-Lahad E, Lahad A (2014) Population-based screening for BRCA1 and BRCA2: 2014 Lasker Award. JAMA 312(11):1091–1092
12. NCCN (National Comprehensive Cancer Network) (2022) Clinical practice guidelines in oncology for genetic/familial high-risk assessment: breast and ovarian vers 1
13. Bernstein-Molho R, Laitman Y, Galper S, Jacobson G, Boursi B, Gal-Yam EN et al (2021) Locoregional treatments and ipsilateral breast cancer recurrence rates in BRCA1/2 mutation carriers. Int J Radiat Oncol Biol Phys 109(5):1332–1340
14. Burstein HJ, Curigliano G, Thürlimann B, Weber WP, Poortmans P, Regan MM et al (2021) Customizing local and systemic therapies for women with early breast cancer: the St. Gallen International Consensus Guidelines for treatment of early breast cancer 2021. Ann Oncol 32(10):1216–1235
15. Apostolou P, Fostira F (2013) Hereditary breast cancer: the era of new susceptibility genes. Biomed Res Int 2013:747318
16. Chen S, Parmigiani G (2007) Meta-analysis of BRCA1 and BRCA2 penetrance. J Clin Oncol 25(11):1329–1333
17. Lord CJ, Ashworth A (2012) The DNA damage response and cancer therapy. Nature 481(7381):287–294
18. van der Groep P, van der Wall E, van Diest PJ (2011) Pathology of hereditary breast cancer. Cell Oncol (Dordr) 34(2):71–88
19. Metcalfe KA, Poll A, Royer R, Llacuachaqui M, Tulman A, Sun P et al (2010) Screening for founder mutations in BRCA1 and BRCA2 in unselected Jewish women. J Clin Oncol 28(3): 387–391
20. Ha SM, Chae EY, Cha JH, Kim HH, Shin HJ, Choi WJ (2017) Association of BRCA mutation types, imaging features, and pathologic findings in patients with breast cancer with BRCA1 and BRCA2 mutations. AJR Am J Roentgenol 209(4):920–928

21. Elezaby M, Lees B, Maturen KE, Barroilhet L, Wisinski KB, Schrager S et al (2019) Mutation carriers: breast and ovarian cancer screening guidelines and imaging considerations. Radiology 291(3):554–569
22. Riis ML (2021) Management of patients with BRCA mutation from the point of view of a breast surgeon. Ann Med Surg (Lond) 65:102311
23. Hartmann LC, Schaid DJ, Woods JE, Crotty TP, Myers JL, Arnold PG et al (1999) Efficacy of bilateral prophylactic mastectomy in women with a family history of breast cancer. N Engl J Med 340(2):77–84
24. Domchek SM, Friebel TM, Singer CF, Evans DG, Lynch HT, Isaacs C et al (2010) Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. JAMA 304(9):967–975
25. Rebbeck TR, Friebel T, Lynch HT, Neuhausen SL, van't Veer L, Garber JE et al (2004) Bilateral prophylactic mastectomy reduces breast cancer risk in BRCA1 and BRCA2 mutation carriers: the PROSE Study Group. J Clin Oncol 22(6):1055–1062
26. Meijers-Heijboer H, van Geel B, van Putten WL, Henzen-Logmans SC, Seynaeve C, Menke-Pluymers MB et al (2001) Breast cancer after prophylactic bilateral mastectomy in women with a BRCA1 or BRCA2 mutation. N Engl J Med 345(3):159–164
27. Heemskerk-Gerritsen BAM, Jager A, Koppert LB, Obdeijn AI, Collée M, Meijers-Heijboer HEJ et al (2019) Survival after bilateral risk-reducing mastectomy in healthy BRCA1 and BRCA2 mutation carriers. Breast Cancer Res Treat 177(3):723–733
28. Rocco N, Montagna G, Criscitiello C, Nava MB, Privitera F, Taher W et al (2021) Nipple sparing mastectomy as a risk-reducing procedure for BRCA-mutated patients. Genes (Basel) 12(2):253
29. Nomikos A, Husain EA, Graham AD (2020) Occult disease in reduction mammoplasties and prophylactic mastectomies. Breast J 26(4):691–696
30. Kaidar-Person O, Boersma LJ, Poortmans P, Sklair-Levy M, Offersen BV, Cardoso MJ et al (2020) Residual glandular breast tissue after mastectomy: a systematic review. Ann Surg Oncol 27(7):2288–2296
31. van Verschuer VM, Maijers MC, van Deurzen CH, Koppert LB (2015) Oncological safety of prophylactic breast surgery: skin-sparing and nipple-sparing versus total mastectomy. Gland Surg 4(6):467–475
32. Bresser PJ, Seynaeve C, Van Gool AR, Brekelmans CT, Meijers-Heijboer H, van Geel AN et al (2006) Satisfaction with prophylactic mastectomy and breast reconstruction in genetically predisposed women. Plast Reconstr Surg 117(6):1675–1682. discussion 83-4
33. Co M, Liu T, Leung J, Li CH, Tse T, Wong M et al (2020) Breast conserving surgery for BRCA mutation carriers—a systematic review. Clin Breast Cancer 20(3):e244–ee50
34. Trombetta MG, Dragun A, Mayr NA, Pierce LJ (2020) ASTRO radiation therapy summary of the ASCO-ASTRO-SSO guideline on management of hereditary breast cancer. Pract Radiat Oncol 10(4):235–242
35. Basu NN, Barr L, Ross GL, Evans DG (2015) Contralateral risk-reducing mastectomy: review of risk factors and risk-reducing strategies. Int J Surg Oncol 2015:901046
36. Basu NN, Ingham S, Hodson J, Lalloo F, Bulman M, Howell A et al (2015) Risk of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers: a 30-year semi-prospective analysis. Familial Cancer 14(4):531–538
37. Metcalfe K, Gershman S, Lynch HT, Ghadirian P, Tung N, Kim-Sing C et al (2011) Predictors of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers. Br J Cancer 104(9):1384–1392
38. Metcalfe K, Lynch HT, Ghadirian P, Tung N, Kim-Sing C, Olopade OI et al (2011) Risk of ipsilateral breast cancer in BRCA1 and BRCA2 mutation carriers. Breast Cancer Res Treat 127(1):287–296
39. van den Broek AJ, Schmidt MK, van't Veer LJ, Oldenburg HSA, Rutgers EJ, Russell NS et al (2019) Prognostic impact of breast-conserving therapy versus mastectomy of BRCA1/

2 mutation carriers compared with noncarriers in a consecutive series of young breast cancer patients. Ann Surg 270(2):364–372
40. Chiba A, Hoskin TL, Hallberg EJ, Cogswell JA, Heins CN, Couch FJ et al (2016) Impact that timing of genetic mutation diagnosis has on surgical decision making and outcome for BRCA1/BRCA2 mutation carriers with breast cancer. Ann Surg Oncol 23(10):3232–3238
41. Terkelsen T, Rønning H, Skytte AB (2020) Impact of genetic counseling on the uptake of contralateral prophylactic mastectomy among younger women with breast cancer. Acta Oncol 59(1):60–65
42. Larsen MJ, Thomassen M, Gerdes AM, Kruse TA (2014) Hereditary breast cancer: clinical, pathological and molecular characteristics. Breast Cancer (Auckl) 8:145–155
43. Lakhani SR, Van De Vijver MJ, Jacquemier J, Anderson TJ, Osin PP, McGuffog L et al (2002) The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p53 in patients with mutations in BRCA1 and BRCA2. J Clin Oncol 20(9):2310–2318
44. Mavaddat N, Barrowdale D, Andrulis IL, Domchek SM, Eccles D, Nevanlinna H et al (2012) Pathology of breast and ovarian cancers among BRCA1 and BRCA2 mutation carriers: results from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). Cancer Epidemiol Biomark Prev 21(1):134–147
45. Vargas AC, Da Silva L, Lakhani SR (2010) The contribution of breast cancer pathology to statistical models to predict mutation risk in BRCA carriers. Familial Cancer 9(4):545–553
46. Foulkes WD, Brunet JS, Stefansson IM, Straume O, Chappuis PO, Bégin LR et al (2004) The prognostic implication of the basal-like (cyclin E high/p27 low/p53+/glomeruloid-microvascular-proliferation+) phenotype of BRCA1-related breast cancer. Cancer Res 64(3):830–835
47. Copson ER, Maishman TC, Tapper WJ, Cutress RI, Greville-Heygate S, Altman DG et al (2018) Germline BRCA mutation and outcome in young-onset breast cancer (POSH): a prospective cohort study. Lancet Oncol 19(2):169–180
48. Welcsh PL, Owens KN, King MC (2000) Insights into the functions of BRCA1 and BRCA2. Trends Genet 16(2):69–74
49. Garutti M, Pelizzari G, Bartoletti M, Malfatti MC, Gerratana L, Tell G et al (2019) Platinum salts in patients with breast cancer: a focus on predictive factors. Int J Mol Sci 20(14):3390
50. Byrski T, Huzarski T, Dent R, Marczyk E, Jasiowka M, Gronwald J et al (2014) Pathologic complete response to neoadjuvant cisplatin in BRCA1-positive breast cancer patients. Breast Cancer Res Treat 147(2):401–405
51. Sikov WM, Berry DA, Perou CM, Singh B, Cirrincione CT, Tolaney SM et al (2015) Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once-per-week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (Alliance). J Clin Oncol 33(1):13–21
52. Tutt A, Tovey H, Cheang MCU, Kernaghan S, Kilburn L, Gazinska P et al (2018) Carboplatin in BRCA1/2-mutated and triple-negative breast cancer BRCAness subgroups: the TNT Trial. Nat Med 24(5):628–637
53. Boughey J, Kalari K, Suman VJ, McLaughlin SA, Moreno-Aspitia A, Moyer AM et al (2016) Abstract SABCS P3-07-29: role of germline BRCA status and tumor homologous recombination (HR) deficiency in response to neoadjuvant weekly paclitaxel followed by antracycline-based chemotherapy. Cancer Res 76:3–7
54. Von Minckwitz G, Hahnen E, Fasching PA, Hauke J, Schneeweiss A, Rezai CSM et al (2014) ASCO annual meeting: pathological complete response (pCR) after carboplatin-containing neoadjuvant chemotherapy in patients with germline BRCA (gBRCA) mutation and triple negative breast cancer: results from GeparSixto. J Clin Oncol 32(15):1005–1005
55. Bayraktar S, Zhou JZ, Bassett R, Gutierrez Barrera AM, Layman RM, Valero V et al (2020) Clinical outcome and toxicity from taxanes in breast cancer patients with BRCA1 and BRCA2 pathogenic germline mutations. Breast J 26(8):1572–1582

56. Walsh CS (2015) Two decades beyond BRCA1/2: homologous recombination, hereditary cancer risk and a target for ovarian cancer therapy. Gynecol Oncol 137(2):343–350
57. Robson ME, Tung N, Conte P, Im SA, Senkus E, Xu B et al (2019) OlympiAD final overall survival and tolerability results: olaparib versus chemotherapy treatment of physician's choice in patients with a germline BRCA mutation and HER2-negative metastatic breast cancer. Ann Oncol 30(4):558–566
58. Litton JK, Rugo HS, Ettl J, Hurvitz SA, Gonçalves A, Lee KH et al (2018) Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. N Engl J Med 379(8): 753–763
59. Tutt ANJ, Garber JE, Geyer CE (2021) Adjuvant olaparib in BRCA-mutated breast cancer. N Engl J Med 385(15):1440
60. Diéras V, Han HS, Kaufman B, Wildiers H, Friedlander M, Ayoub JP et al (2020) Veliparib with carboplatin and paclitaxel in BRCA-mutated advanced breast cancer (BROCADE3): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 21(10):1269–1282
61. Vinayak S, Tolaney SM, Schwartzberg L, Mita M, McCann G, Tan AR et al (2019) Open-label clinical trial of niraparib combined with pembrolizumab for treatment of advanced or metastatic triple-negative breast cancer. JAMA Oncol 5(8):1132–1140
62. Domchek SM, Postel-Vinay S, Im SA, Park YH, Delord JP, Italiano A et al (2020) Olaparib and durvalumab in patients with germline BRCA-mutated metastatic breast cancer (MEDIOLA): an open-label, multicentre, phase 1/2, basket study. Lancet Oncol 21(9): 1155–1164
63. Sønderstrup IMH, Jensen MB, Ejlertsen B, Eriksen JO, Gerdes AM, Kruse TA et al (2019) Evaluation of tumor-infiltrating lymphocytes and association with prognosis in BRCA-mutated breast cancer. Acta Oncol 58(3):363–370
64. Sabatier R, Finetti P, Mamessier E, Adelaide J, Chaffanet M, Ali HR et al (2015) Prognostic and predictive value of PDL1 expression in breast cancer. Oncotarget 6(7):5449–5464
65. Toss A, Molinaro E, Venturelli M, Domati F, Marcheselli L, Piana S et al (2020) BRCA detection rate in an Italian cohort of luminal early-onset and triple-negative breast cancer patients without family history: when biology overcomes genealogy. Cancers (Basel) 12(5): 1252
66. Schmid P, Cortes J, Pusztai L, McArthur H, Kümmel S, Bergh J et al (2020) Pembrolizumab for early triple-negative breast cancer. N Engl J Med 382(9):810–821
67. Nichols KE, Malkin D, Garber JE, Fraumeni JF, Li FP (2001) Germ-line p53 mutations predispose to a wide spectrum of early-onset cancers. Cancer Epidemiol Biomark Prev 10(2):83–87
68. Evans DG, Birch JM, Ramsden RT, Sharif S, Baser ME (2006) Malignant transformation and new primary tumours after therapeutic radiation for benign disease: substantial risks in certain tumour prone syndromes. J Med Genet 43(4):289–294
69. Birch JM (1994) Li-Fraumeni syndrome. Eur J Cancer 30A(13):1935–1941
70. Bougeard G, Brugières L, Chompret A, Gesta P, Charbonnier F, Valent A et al (2003) Screening for TP53 rearrangements in families with the Li-Fraumeni syndrome reveals a complete deletion of the TP53 gene. Oncogene 22(6):840–846
71. Rich TA, Woodson AH, Litton J, Arun B (2015) Hereditary breast cancer syndromes and genetic testing. J Surg Oncol 111(1):66–80
72. Masciari S, Dillon DA, Rath M, Robson M, Weitzel JN, Balmana J et al (2012) Breast cancer phenotype in women with TP53 germline mutations: a Li-Fraumeni syndrome consortium effort. Breast Cancer Res Treat 133(3):1125–1130
73. Birch JM, Alston RD, McNally RJ, Evans DG, Kelsey AM, Harris M et al (2001) Relative frequency and morphology of cancers in carriers of germline TP53 mutations. Oncogene 20(34):4621–4628
74. Kasper E, Angot E, Colasse E, Nicol L, Sabourin JC, Adriouch S et al (2018) Contribution of genotoxic anticancer treatments to the development of multiple primary tumours in the context of germline TP53 mutations. Eur J Cancer 101:254–262

75. Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z et al (1997) Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet 16(1):64–67
76. Nelen MR, Padberg GW, Peeters EA, Lin AY, van den Helm B, Frants RR et al (1996) Localization of the gene for Cowden disease to chromosome 10q22–23. Nat Genet 13(1): 114–116
77. Herman GE, Butter E, Enrile B, Pastore M, Prior TW, Sommer A (2007) Increasing knowledge of PTEN germline mutations: two additional patients with autism and macrocephaly. Am J Med Genet A 143A(6):589–593
78. Pilarski R, Burt R, Kohlman W, Pho L, Shannon KM, Swisher E (2013) Cowden syndrome and the PTEN hamartoma tumor syndrome: systematic review and revised diagnostic criteria. J Natl Cancer Inst 105(21):1607–1616
79. Thull DL, Vogel VG (2004) Recognition and management of hereditary breast cancer syndromes. Oncologist 9(1):13–24
80. Komiya T, Blumenthal GM, DeChowdhury R, Fioravanti S, Ballas MS, Morris J et al (2019) A pilot study of sirolimus in subjects with cowden syndrome or other syndromes characterized by germline mutations in *PTEN*. Oncologist 24(12):1510–e265
81. Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A et al (2007) PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. Nat Genet 39(2): 165–167
82. Erkko H, Dowty JG, Nikkilä J, Syrjäkoski K, Mannermaa A, Pylkäs K et al (2008) Penetrance analysis of the PALB2 c.1592delT founder mutation. Clin Cancer Res 14(14):4667–4671
83. Heikkinen T, Kärkkäinen H, Aaltonen K, Milne RL, Heikkilä P, Aittomäki K et al (2009) The breast cancer susceptibility mutation PALB2 1592delT is associated with an aggressive tumor phenotype. Clin Cancer Res 15(9):3214–3222
84. Dorling L, Carvalho S, Allen J, González-Neira A, Luccarini C, Wahlström C et al (2021) Breast cancer risk genes—association analysis in more than 113,000 women. N Engl J Med 384(5):428–439
85. Tung NM, Robson ME, Ventz S, Santa-Maria CA, Nanda R, Marcom PK et al (2020) TBCRC 048: phase II study of olaparib for metastatic breast cancer and mutations in homologous recombination-related genes. J Clin Oncol 38(36):4274–4282
86. van Lier MG, Wagner A, Mathus-Vliegen EM, Kuipers EJ, Steyerberg EW, van Leerdam ME (2010) High cancer risk in Peutz-Jeghers syndrome: a systematic review and surveillance recommendations. Am J Gastroenterol 105(6):1258–1264. author reply 65
87. Launonen V (2005) Mutations in the human LKB1/STK11 gene. Hum Mutat 26(4):291–297
88. Aretz S, Stienen D, Uhlhaas S, Loff S, Back W, Pagenstecher C et al (2005) High proportion of large genomic STK11 deletions in Peutz-Jeghers syndrome. Hum Mutat 26(6):513–519
89. Pharoah PD, Antoniou A, Bobrow M, Zimmern RL, Easton DF, Ponder BA (2002) Polygenic susceptibility to breast cancer and implications for prevention. Nat Genet 31(1):33–36
90. Stracker TH, Usui T, Petrini JH (2009) Taking the time to make important decisions: the checkpoint effector kinases Chk1 and Chk2 and the DNA damage response. DNA Repair (Amst) 8(9):1047–1054
91. Consortium CBCC-C (2004) CHEK2*1100delC and susceptibility to breast cancer: a collaborative analysis involving 10,860 breast cancer cases and 9,065 controls from 10 studies. Am J Hum Genet 74(6):1175–1182
92. Han FF, Guo CL, Liu LH (2013) The effect of CHEK2 variant I157T on cancer susceptibility: evidence from a meta-analysis. DNA Cell Biol 32(6):329–335
93. Mellemkjaer L, Dahl C, Olsen JH, Bertelsen L, Guldberg P, Christensen J et al (2008) Risk for contralateral breast cancer among carriers of the CHEK2*1100delC mutation in the WECARE Study. Br J Cancer 98(4):728–733
94. Adank MA, Jonker MA, Kluijt I, van Mil SE, Oldenburg RA, Mooi WJ et al (2011) CHEK2*1100delC homozygosity is associated with a high breast cancer risk in women. J Med Genet 48(12):860–863

95. Easton DF, Pharoah PD, Antoniou AC, Tischkowitz M, Tavtigian SV, Nathanson KL et al (2015) Gene-panel sequencing and the prediction of breast-cancer risk. N Engl J Med 372(23): 2243–2257
96. Shiloh Y (2006) The ATM-mediated DNA-damage response: taking shape. Trends Biochem Sci 31(7):402–410
97. Bernstein JL, Teraoka S, Southey MC, Jenkins MA, Andrulis IL, Knight JA et al (2006) Population-based estimates of breast cancer risks associated with ATM gene variants c.7271T>G and c.1066-6T>G (IVS10-6T>G) from the Breast Cancer Family Registry. Hum Mutat 27(11):1122–1128
98. Renwick A, Thompson D, Seal S, Kelly P, Chagtai T, Ahmed M et al (2006) ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. Nat Genet 38(8): 873–875
99. Jerzak KJ, Mancuso T, Eisen A (2018) Ataxia-telangiectasia gene. Curr Oncol 25(2):e176–ee80
100. Ratajska M, Antoszewska E, Piskorz A, Brozek I, Borg Å, Kusmierek H et al (2012) Cancer predisposing BARD1 mutations in breast-ovarian cancer families. Breast Cancer Res Treat 131(1):89–97
101. Stacey SN, Sulem P, Johannsson OT, Helgason A, Gudmundsson J, Kostic JP et al (2006) The BARD1 Cys557Ser variant and breast cancer risk in Iceland. PLoS Med 3(7):e217
102. Ahmed M, Lalloo F, Evans DG (2009) Update on genetic predisposition to breast cancer. Expert Rev Anticancer Ther 9(8):1103–1113
103. Klein HL (2008) The consequences of Rad51 overexpression for normal and tumor cells. DNA Repair (Amst) 7(5):686–693
104. Zhou GW, Hu J, Peng XD, Li Q (2011) RAD51 135G>C polymorphism and breast cancer risk: a meta-analysis. Breast Cancer Res Treat 125(2):529–535
105. Thompson ER, Boyle SE, Johnson J, Ryland GL, Sawyer S, Choong DY et al (2012) Analysis of RAD51C germline mutations in high-risk breast and ovarian cancer families and ovarian cancer patients. Hum Mutat 33(1):95–99
106. Osher DJ, De Leeneer K, Michils G, Hamel N, Tomiak E, Poppe B et al (2012) Mutation analysis of RAD51D in non-BRCA1/2 ovarian and breast cancer families. Br J Cancer 106(8): 1460–1463
107. Park DJ, Lesueur F, Nguyen-Dumont T, Pertesi M, Odefrey F, Hammet F et al (2012) Rare mutations in XRCC2 increase the risk of breast cancer. Am J Hum Genet 90(4):734–739
108. Sharif S, Moran A, Huson SM, Iddenden R, Shenton A, Howard E et al (2007) Women with neurofibromatosis 1 are at a moderately increased risk of developing breast cancer and should be considered for early screening. J Med Genet 44(8):481–484
109. Wang X, Levin AM, Smolinski SE, Vigneau FD, Levin NK, Tainsky MA (2012) Breast cancer and other neoplasms in women with neurofibromatosis type 1: a retrospective review of cases in the Detroit metropolitan area. Am J Med Genet A 158A(12):3061–3064
110. Attwooll CL, Akpinar M, Petrini JH (2009) The mre11 complex and the response to dysfunctional telomeres. Mol Cell Biol 29(20):5540–5551
111. Heikkinen K, Rapakko K, Karppinen SM, Erkko H, Knuutila S, Lundán T et al (2006) RAD50 and NBS1 are breast cancer susceptibility genes associated with genomic instability. Carcinogenesis 27(8):1593–1599
112. Win AK, Lindor NM, Jenkins MA (2013) Risk of breast cancer in Lynch syndrome: a systematic review. Breast Cancer Res 15(2):R27
113. Stoll J, Rosenthal E, Cummings S, Willmott J, Bernhisel R, Kupfer SS (2020) No evidence of increased risk of breast cancer in women with lynch syndrome identified by multigene panel testing. JCO Precis Oncol 4:51–60
114. Harkness EF, Barrow E, Newton K, Green K, Clancy T, Lalloo F et al (2015) Lynch syndrome caused by MLH1 mutations is associated with an increased risk of breast cancer: a cohort study. J Med Genet 52(8):553–556

Part VII

Patient Advocacy

My Hereditary Gastric Cancer

24

Maria Troina

I have decided to tell my story in a different way, I do not want to list the problems related to nutrition or to the acceptance of living without a stomach. I want to tell you about Maria, her emotions, her joys, her fears, her defeats and her victories.

My name is Maria Troina and this year I will turn 45. I live in a village halfway between Rimini and Cesena, in Emilia Romagna. I have two children: Marco born in 2009 and Martina born in 2012. In September my husband and I will celebrate our 25th anniversary of marriage. I lived a painful childhood because at the age of 9, in 1987, my mother passed away, she was 32 years old. I am the eldest of three siblings, our childhood was marked by the loss of our mother. I got married to the love of my life, the one that when you first see it closes your stomach and stops your hunger, you feel butterflies in your stomach and your heart beats faster, I was 14, he was just turning 18. Over the years we have taken and left each other several times but decided that as soon as I finished school and as soon as possible for him we would get married (he is a *Carabiniere* and then they had to spend 4 years from the voluntary stop to get married, or have turned 24). I lived a happy life, as soon as we got married, we moved to another region (Marche) because of my husband's job. After a few years we returned home, we bought a small apartment, but I didn't feel like having children, only the hypothesis that they could go through what I lived, growing up without a mother, frightened me; jokingly, I used to say: "No, Mattia, you better be the cheerful widower, than the desperate father." Later, armed with new courage and the desire to complete our family, at the age of 32, after 12 years of marriage, in 2009 Marco was born, and in 2012 Martina. In 2013 I discovered that I am a carrier of the CDH1 genetic mutation, and that the cause of my mother's death

In this chapter the author describes a personal experience with the diagnosis, treatment and care of hereditary cancer.

M. Troina (✉)
Cesena, Italy

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_24

Fig. 24.1 Maria Troina and Prof. Franco Roviello

was due to that mutation; my biggest nightmare became reality: my children had very high chances of living my same childhood, I felt this as a curse. After assimilating the news, I researched about this mutation, gathered information and took my life in my hands: I wanted to be the one in charge, I wanted to be able to do everything I could to continue living! I joined a Facebook group: *Vivere senza stomaco, si può* (to live without a stomach is possible) looking for information and contacts. A few days before Christmas, I wrote my story on the fb group and I got in contact with Professor Franco Roviello (Fig. 24.1), my savior, the one who reassured me, protected me and performed the surgery. We met, we did all the necessary checks and in 2015 I got the surgery, among the first ones in Italy to receive this kind of intervention on a preventive basis. I entered the hospital in healthy conditions and I came out without my stomach: it was not easy; it was not taken for granted. I want to live and I want to be an example for my children, if they were to be positive to the same genetic mutation, I want to show them that life can still smile at you and that it can be faced (there is a 50% chance of having transmitted the mutation to my children). I've been criticized for my choice, I've been supported, and pitied, but life is mine and only I can decide what's best for me.

I am the fruit of the love and dreams of two young emigrants who met at the Italian club in Wolfsburg (Germany) in 1975.

So, let's take a small step back.

In the mid-60s, my paternal grandfather left with a cardboard suitcase tied with a string for Germany, he left his beloved Sicily, Catania, to be precise, in search of work, leaving his wife his five sons and his daughter at home.

Arriving in Germany, he lived for a year in the slums, where the Italians were "housed," all males, work, work and a lot of melancholy. After more than a year, he was given public housing, and so the whole family moved to Germany. Even today it moves me to hear the stories of my father and his brothers from the sun and the pranks in Sicily, to Germany: cold, "impaled," the shy Germans do not smile, the children did not scream, they did not get dirty, they did not go from house to house. A similar story for my maternal family: we have a different village of origin: Capizzi, in the mountains near Messina, and a different set of siblings: five daughters and a son. The years passed, the integration took place quickly, they learned the language and attended school, little by little even the entertainment and parties returned to animate their everyday life. Italian clubs were born, where it was customary to spend party evenings and Saturday evenings. It was on the occasion of one of these parties that my parents met, a sympathy was born and shortly thereafter they got married. The sympathy was so strong that three brothers of my paternal family married three sisters of my maternal family: two families got strongly linked in a bond that certainly helped us to face all the pain and the obstacles that life had decided to reserve for us.

In 1977, I was born and in 1979 my brother Angelo; despite a good job, friends and family, my parents decided to return to Italy in 1983 to let me start the school at my "home-country," the decision was to buy a house in Cesena, in Emilia Romagna, because the much-loved Sicily, although beautiful, was not able to guarantee a working future for my father, and consequently there were no job prospects for us children.

How much love, how much happiness? In 1986, my parents decided to expand the family and my little brother Salvatore was born. In the meantime, my father's brother who had married my mother's sister also moved a few steps away from us.

My mother died at the age of 32 of stomach cancer (1987).

In April 2013, my mom's younger sister (my mother's third sister who married my father's third brother), at the age of 43, found out she had stomach cancer. She lived in Germany; she was a woman who performed annual checkups. Fortunately, the German doctor she was in contact with, analyzing the family history, came up with the idea of talking to her about the mutation of the CDH1 gene Ekaterina; a mutation he had casually read about a few weeks earlier in a scientific journal. He talks about it with her, and after her consent, he books her at the University Clinic of Hanover for an exam to see if she is a carrier of the CDH1 gene mutation, she performs the test, the result arrives, she is positive.

I contacted a geneticist in Italy and we all ran the test in the family, I am positive. The geneticist tells me: well now we are stronger, we do a mammogram every year and a gastroscopy every 10/12 months. This type of mutation gives an 80/90% chance of developing stomach cancer, and a 50–70% chance of developing breast cancer. I inform the geneticist that in Germany, in case of positivity, they suggest the removal of the stomach as a precaution, that it is a rare mutation and that the type of tumor that it develops is under the mucosa, so once identified with gastroscopy it is too late, but her suggestion remains the same. I contacted my aunt's German doctors, and they confirm that on their part the advice is that of surgery.

On April 16 of 2015 I had surgery in Siena. My surgeon, Professor Franco Roviello, gives me the opportunity to be reborn.

There are days I find myself reflecting on how my life has changed since April 16, 2015, how Maria of that time did not have anything in common with today's Mary. Not from a physical point of view but psychological! I had to fight with food, with my head and my body! I suffered without being able to "fight" what my body imposed on me and my head did not accept. But there is always a but, especially for those who are positive, those who always see the glass as half full, those who give something to the world, those that try to find something good from a negative event, I feel and am happy!

I learned to appreciate the little things, I learned to "listen to myself" I re-assigned priorities based on my being, I stopped hanging out with people who didn't make me feel good. I learned not to make judgments without knowing the facts, even if I don't agree with it, I try to understand a vision that's different from mine. I am trying to love myself, enjoying life, with all the limits that my body imposes on me, without living it as a privation.

Thanks to these limits, I have more time to look at the sky, the stars, children playing, the cat arguing with flies.

You can live without a stomach.

There are difficult days, sorrows, thoughts and pain, for us who are already "fragile" on our own, who every day try to conquer and maintain our balance it's even more difficult, let's remember to be happy and to enjoy the little things, life is beautiful, let's never forget it!

In moments of sadness and joy I go look for the message of my dear Fausto Servizi, whom I met the week before the surgery thanks to the fb group I belong to: "You can live without a stomach" supported me, guided and reassured me, it is difficult to explain how it is possible to be able to feel so connected and love someone so well in such a short time. For me he was a spiritual father, unfortunately he left us, but I always carry him in my heart; in every achievement and every goal I think of him and I know he would be proud of me! THANKS FAUSTO, I was able to face everything with a little more serenity thanks to you! I want to share the message he sent me the night before the surgery "it is said that the prince of Condé slept soundly the night before the battle" The following morning, he won it!

I came to weigh 42 kg, (I am 170 cm tall) and it was very hard, I had to learn to do the intramuscular punctures myself, I am afraid of needles.

Doberin, it hurts terribly, so I decided that I will manage my pain my way!

I have moments when the mood is not the best, being close to me is not easy. Sometimes the frailties take over.

We are inspired by eagles. Eagles live 70 years, but at 40 they have to make a difficult decision, their nails become so long and flexible that they cannot hold back the prey they feed on. The long-pointed beak curves too much against the chest and is no longer useful.

Their wings have aged and are heavy based on the large size of their feathers, thus their flight becomes very difficult.

The eagle has two alternatives: abandon herself and die, or go through a painful renewal process, which consists of flying to a nest in the mountains near a wall, as it is safer. The eagle starts hitting the wall with its beak with great force until it is detached.

Then it will wait for the growth of a new beak, with which it will peel off its old nails one by one. When the new claws begin to hatch, it will begin to pluck its worn-out feathers. After all those long and painful 5 months of wounds, scars and regrowth, it manages to keep its famous flight of renewal, rebirth and celebration for getting to be alive another 30 years. In our life to continue a flight of victory many times we have to take shelter for some time and start a process of renewal. We have to get rid of customs, traditions and memories whose weight prevents us from moving forward. Only free from the past can we exploit the precious result that a renewal always brings us. Renewing within involves putting the mental world in order, discarding the memories of frustrating or painful events to be left alone with the experience of what we have learned. In order to renew ourselves and take flight, we must know ourselves, know who we are, what our potential is and where we want to go. It is not necessary to adapt to the problem; there is a chance to get rid of it. But the road is a bit difficult, the road is a challenge. It's your choice. We follow the route of the eagles: Always standing, always ahead. Since after the intervention I have become an active part of the association "Living without a stomach is possible" and today, with great honor, I am the vice president. I am very grateful to Claudia Santangelo for her commitment, for what she has done and is doing for the patients, and for me it is an honor to be able to bear my testimony and represent the association to which I belong, so that the voice and the needs of patients are always in first place. Seeing the emotion and "feeling" the support of the public is an experience that I will always carry in my heart (Fig. 24.2).

Unfortunately, in addition to the difficult condition we live in, the bureaucracy does not help us, I am young and I do not want to lose my working dignity, but unfortunately the State does not allows us to live peacefully to be able to work and take care of ourselves, I would not be able to work 8 h a day, because after eating something I need to lie down, if I eat I feel bad, I run the risk of spending a lot of time in the bathroom, therefore the possibility of working part-time allows me to be an active part of society and financial help for my family.

Fig. 24.2 Maria Troina at the Barcellona Digestive Cancer Europa 2019

I am called on a regular basis by the *INPS* for the review of the disability that has been recognized to me, so the weeks preceding the visit are always of tension and concern.

Without the stomach we live in a condition that is not "normal" but our game has to be played in front of that commission. For many like me that do not understand, or the "oh well now it's okay" will affect everything. I washed myself, without caring to much about the details, but not unkempt, I kept my fingers crossed all the time. I used a firm but calm tone. The decision of that commission will give me the opportunity not to lose my working dignity, or it will bury me . . . when leaving I breathed a sigh of relief and I started to cry. Because it is not fair. An institution intended for the protection of the weakest should not let us experience this: keeping our fingers crossed while not sleeping at night always in between hope and fear!

Hope that the commission realizes that everything is difficult and that surviving it does not mean that everything is ok. Losing the inability certification might imply the loss of the right to a part-time employment, thus having to give up work. Less money, another sinking for us! Yes, because in addition to the thought we also weigh on the economic condition of the family.

I got in the car, I cried, hugged my husband, and I said. But life is strange! Thanks to what happened to me I have revised, revolutionized my priorities. I've always been a positive woman!

I lost my stomach on the street. I will always be very thin, but now I appreciate life. Every day is an important day, every season has its beauty, every breath makes me feel alive! Happy to be happy with what I can do.

DO IT.

Wear that dress too tight.

Let your hair down.

Get up and dance.

Find reasons to laugh.

Make love.

Create something beautiful.

Speak out.

Recognize your worth.

Don't apologize for your magic anymore and stop hiding your light.

Love yourself.

Forgive yourself.

Make room for the unexpected.

Stop waiting for the right time, do it now.

Ignore what people think of you.

Because, at the end, you will have to answer for all the words you didn't say, the people you didn't love, the things you didn't do and the places you didn't go.

Do it now.

I stopped going out to eat because several times I passed out and had to be taken to the hospital. One evening I promised that I would go to a party. I got ready, made up. and went, I did it crawling because I wanted everything except to go out, not to mention that I had no strength. . . . It was a party dedicated to a disco that closed in 1992. I attended the closing evening that year, with the boy who would later become my husband. My whole life passed in front of me, I appreciated the past, I remembered it with nostalgia, emotion, happiness and serenity! Serenity! I made my dreams come true, I lived a happy life, with the love of my life, so much happiness, so much fear, so many disappointments, but always together with every obstacle in life, always together, always united and ready to support each other! I retraced my life, I am aware of what happened to me, of my great strength, but above all of my fragility!! Strength and fragility together, not one or the other, both to achieve a balance! I realized that I am alive thanks to my choices! Thanks to my courage, I danced, hugged my husband and enjoyed myself with new and old friends, smiling and shedding a few tears because I was there, it was not obvious, thanks to my choices and the support of my husband I was there! And not the memory of friends and a photo on someone's shelf! Today more than other days I am aware that life is unique, one must be lived, taken in bites, enjoyed, without too many ifs and buts, do not postpone the beautiful thoughts, it will not be done tomorrow! A day that started normally turned into a tired, frustrating day, it ended with a heart full of joy and great awareness! However, it goes, it will be a success! Because I live! It can't rain forever; I'm waiting for the sun. At midnight I congratulated my son by

dancing and singing with him holding tightly tight! I celebrated my 10th birthday without my mom, fate had the same in store for him, but I arrived a moment earlier!

After the surgery it was hard, I wanted to see the light at the bottom of the tunnel, I no longer felt the flavors, they had changed, what I loved disgusted me, being able to eat bread and pasta, go from 56 kg from the day of the intervention to weight 44 kg, (I am 1.71 m tall) and seeing on the scale that I was losing weight every day was hard difficult, a dream to be able to eat everything without going into diarrhea.

I wanted to take back my life, I would like my husband to regain some serenity and time for himself. We have two children who fortunately helped us not to despair for the obstacles we had to face. Fortunately, they have their needs and their liveliness; and their needs do not look at anything, they just want to play and this forced me to react. Thanks to my family, especially to my aunt Rosa and uncle Franco, and to my cousin, who is like a sister to me, my husband and I managed to do it. They are always present, always close to me and my family. I will never forget my uncle who, the day before the surgery, came to me and told me to only think of myself because the children were in good hands. They gave me the opportunity to face everything with the utmost serenity, and to live without a stomach is possible!

The year 2015 scared me, terrified me, made me cry. I was looking for normal comfort in the posts of my Facebook group. I was lucky, I had surgery in a preventive way, in addition to the surgery they had to undergo all the rest of the chemo ... perhaps the worst part! From the day after the surgery, I had become another person, certainly stronger, but at the same time more fragile. From the awakening after surgery, I would have been different. A new life would have begun for me, the new one scared me, but I was sure that I would have made that life mine from the first second, I felt as if the time available to me before the surgery had been too little, I was not sleeping, continuously thinking about the things that I had to do before because maybe I could no longer do them, take care of my children, my husband, the trips, the dinners, the house. How much fear.

Slowly I learned and understood that we should not cry about what we have lost, but celebrate what we found!!! Life is Beautiful!!

It helps me to remember the seahorse, he has no stomach, so if he can, why shouldn't I do it.

Some days we get discouraged, we don't see the light some days, but we are strong! We find the strength unfounded, and we shine! We know what fear is!

What is happiness!

We are the will to live!

Because you can live without a stomach!

I realized that I no longer think about the fact that I have no stomach, when I feel bad, I think as before the surgery about how to solve the problem, I realize that I am living much better and I am regaining my normality. I feel I can say that:

Today I wear my scar with the awareness that without her I would not be here!!! It didn't make me weaker, more aware; life is one and it deserves to be lived! There is nothing taken for granted! Life is Beautiful!

Fig. 24.3 Maria Troina and her family

In 2017 I felt ready, I booked a flight and together with my son we left, I was traveling for the first time without my stomach while he was on his first trip alone with his mother, destination Berlin.

Life must be bitten! We must remember to chew well! I went back to doing what I loved traveling, playing, cooking (Fig. 24.3).

Hereditary Breast Cancer Syndrome, My Experience

25

Francesca Stella

In 2019, I was 30. I'm a sales manager for a fashion company but I'm also a part-time personal trainer. I could work in office, I could teach in the gym, I could run 10 km, I could party at night, everything in the same day, without any difficulty. I was 30 and feeling stronger than ever. I run a race in April '19 doing my personal best and the day after I had an appointment at IEO for a breast ultrasound. Few months earlier, I noticed a little marble in my right breast, I was sure it was a cyst but I wanted to make sure. During the ultrasound, the radiologist decided to do also a needle aspiration.

Few days after the exam, my medical doctor called me since she had received the medical report: I had breast cancer. I couldn't believe it, I was feeling great, I couldn't realize I was sick. No one in my family had cancer before, I don't smoke, I barely drink a gin&tonic on Fridays, I eat healthy food, I train a lot, since ever (Fig. 25.1). It was impossible to me to find an explanation.

Few weeks after my genetic test shown that I have BRACA1 mutation. Somehow, that was the answer, at least to me.

The cancer was quite big, almost 4 cm, so first step was a neoadjuvant chemotherapy instead of going to surgery straightaway.

Before to start, I even asked "Is chemo that treatment that makes you lose your hair?". I was totally unprepared so I decided to simply follow my doctor's indications. First thing to do was to freeze the eggs in order to start with chemo urgently. Four red chemotherapies and 12 taxol, then the surgery, this was the plan.

I was full of energy when I started, even though I was scared. I didn't want to stop training, I didn't want to look weak or sick to the people, and I didn't want to change

In this chapter the author describes a personal experience with the diagnosis, treatment and care of hereditary cancer.

F. Stella (✉)
Milan, Italy

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_25

Fig. 25.1 Francesca at work

my routine. I bought a wig, an expensive one but no one noticed the difference with my hair. I pretended nothing wrong was happening. After the first chemo, I went to Ibiza for an already-planned weekend away. The flight was a nightmare, I had a terrible nausea but above all I felt scared. Once arrived, the sea view and the feeling of that hippie life relaxed me. I was still be able to run, I was feeling quite ok, I started to lose my hair after 14 days. That day was terrible. I was prepared, I was counting days, I had the perfect wig, but still. . . it was atrocious. I've always been good-looking; I've always had a perfect sporty body. That day the relationship with my body started to change. I felt like I couldn't approach to men anymore or accept a date invitation or anything related to love affair.

Second chemo was 3 weeks after the first and few days before I woke up with my left arm very swollen. The combination of port-a-cath and chemo caused me a thrombosis and I couldn't use the port for the second chemo. I felt powerless, I was doing my best, even more, and I didn't predict this obstacle (Control craze anyone?! 😊). Three remaining red chemo directly in my right arm blood vessel, I couldn't even read a book during the injection but I had no choice, I had to accept it.

My running pace started to get slower but I kept running, working, teaching in the gym, going out with my friends. I kept pretending nothing was going on to the world but my best friends, I needed them to know.

Fourth out of four chemo was at the beginning of August, that was bad. Bad is probably not even enough, for the first time I had to stay 1 week in bed, being able to do nothing. My mom asked me "how do you feel? Which kind of problem do you have?" I didn't even know, I was just feeling barely able to breath, and the hot temperature didn't help. After that week I woke up and I was feeling good again so I took my bike and went out for a 30-km ride. That feeling was incredible, it was freedom to me.

12 taxol, every week. I had to spend a lot of energies already and the path started to look long and hard. First taxol chemo was strong and I didn't expect it. My doctors told me "oh you'll see, taxol is nothing compared to red chemo, the worst is gone." For the first time, I felt demoralized and decided to start a psycho-oncology program. It changed my life. Few weeks after I also decided to post a picture on Instagram a photo of me without wig revealing to the world, I had cancer. I had the confirmation nobody noticed anything for 6 months, people were shocked and I took 2 days to reply to all the love messages received.

My life became easier, people were not expecting the best from me anymore and that was a relief to me, surprisingly.

After chemo, the cancer disappeared but I choose to do the double mastectomy anyway. I used to like my breast, I was scared about the result but, at that point I learned that I had no choice but to hope for the best and trust the doctors. The day of the surgery I was incredibly confident and focus on the real goal: close that chapter. One more time I felt I had underestimated the situation. My body was suffering and so was my mind. Even though I went back to work after 2 weeks, the recovery was slow and painful. But the result was great, I have to admit, I still feel very grateful.

Due to my genetic mutation and the type of cancer I had, my doctors decided to complete my program with 21 radiotherapy. After months of chemo, after surgery, that 30-min radiation everyday looked like a game to me. During my last radiation, I decided to take a year off work and to travel all around the world just trying to enjoy my life. Covid started 1 week after and I had to change my plans one more time. Resiliency and adaptability, lesson learned.

Part VIII

Miscellaneous

β-Hemoglobinopathies and Early Onset of Cancers in Adulthood: Epidemiology in Southeastern Asia and Brunei with Emphasis for Prevention and Treatment

26

Meric A. Altinoz, Francesca Magnoni, Aysel Ozpinar, and Giovanni Corso

Abstract

Despite occurring with lesser incidence in comparison with Western countries, breast cancers (BC) manifest at earlier ages than in the Western World than in Southeast Asia, where thalassemias and hemoglobinopathies are highly prevalent. Cord blood analyses in Singapore revealed that Malays have higher rates of HbE and β-thalassemia than Chinese and Indians. Among Southeast Asian populations, Malays have a worse prognosis for BC with early age of onset. Peculiarly, Brunei has lowest cancer mortality rates among ASEAN countries, yet neurological cancers had the highest percentage of young adult patients. 11p15.5 genomic region includes β-globin genes in the order of 5′-ε–γG–γA–δ–β-3′ and genes which associate with pathogenesis of early age breast cancers and brain tumors such as IGF-2, SLC22A18, H19/Wilms tumor-2, ILK, TSSC3/PHLDA2, p57kip2/CDKN1C, and HRAS. β-Globin genes and tumor-proneness genes at 11p15.5 may possess haplotype interactions and proval of this hypothesis would be important for cancer prediction and prevention. In subjects with thalassemia trait (as shown by rapid and economical tests), cancer screenings may be intensified. The lack of an association in other World regions where hemoglobinopathies are prevalent (such as Middle Asia and Africa) may be due to the different types of hemoglobin mutations leading different types and levels of hemorphins, the splicing products of hemoglobins which regulate immunity.

M. A. Altinoz (✉) · A. Ozpinar
Department of Medical Biochemistry, Acibadem University, Istanbul, Turkey

F. Magnoni
Division of Breast Surgery, European Institute of Oncology, IRCCS, Milan, Italy

G. Corso
Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy

G. Corso et al. (eds.), *Hereditary Gastric and Breast Cancer Syndrome*,
https://doi.org/10.1007/978-3-031-21317-5_26

26.1 Introduction

Despite a huge number of basic and clinical research and significant improvements, cancer remains a leading cause of mortality globally and recent studies emerged showing associations between hemoglobin biochemistry and oncogenesis [1]. Breast cancers that early arise at reproductive ages put a devastating burden on women's health, yet consistent reports about early age occurrence of breast cancer in Southeast Asian countries did not gain sufficient interest in the research community investigating molecular carcinogenesis [2, 3]. Recently, studies appeared about increasing cancer cases in Brunei, which include breast and brain cancers with early age of onset [3, 4]. Here, we focus on the epidemiological feature of early age breast and brain cancer accumulation in Southeast Asian population and in Brunei, particularly. We believe that this would provide novel precious information in understanding molecular etiology of these cancers. These are World regions, where unique β-globin mutation-associated diseases are also very frequent. We propose that cancer-susceptibility gene loci at 11p15.5 may exert haplotype associations with β-globin mutations. To strengthen our proposal, we provided an extensive librarial data; and if this hypothesis could be proven in future, it may lead to important means of cancer prediction and prevention. For instance, patients with certain hemoglobin mutations could be screened for cancer more frequently or intensively.

26.2 Population Structure and Cancer Burden in Southeast Asia and Brunei

Ten countries with very heterogeneous ethnicity constitute Southeast Asia. Malayo-polynesians (Austronesian) live in Malaysia, Brunei, Indonesia, the Philippines, and in various Pacific islands [5]. Indians and Chinese are relatively new inhabitants. The Association of Southeast Asian Nations (ASEAN) is an organization of ten countries: Cambodia, Brunei, Malaysia, Indonesia, Myanmar, Laos, the Philippines, Thailand, Singapore, and Viet Nam [6]. This region stores almost 9% of the global population, including countries with very different socio-economical features, from economic powers like Singapore to poor economies such as Cambodia, Laos, and Myanmar [6]. In 2008, there occurred about 725,000 novel cases of cancer (excluding non-melanoma dermal malignancies) and about 500,000 cancer-associated fatalities in the ASEAN region. In Southeast Asian women, breast cancers were the most frequent malignancies, followed by cervix and colorectal malignancies [6]. In ASEAN region, breast cancers comprise 22% of the newly diagnosed female cancer patients and 15% of the malignancy-related mortalities in 2008. The highest incidence is encountered in Singapore (59.9 per 100,000) and the lowest in Vietnam (15.6 per 100,000) [6]. However, mortality rates per 100,000 peak in Indonesia (36.2) are significantly lower in Singapore (13.6), and lowest in Vietnam [6]. Here, it shall be noted that Malays make up a large portion of the populations in Indonesia and Malays were shown to have higher β-globin mutations in Singapore (6.3% than

2.7% in the Chinese and 0.7% in Indians, respectively) and people of Malay descent with breast cancer were shown to have worse prognosis [7, 8].

Among ASEAN countries, Brunei has the highest GDP per capita about 188 times higher than Myanmar with a population composing of about 66% Malays, 11% Chinese, and 22% "others" made up of foreigners of different nationalities and indigenous people of Borneo [2]. Interesting patterns are witnessed, when investigating incidence and mortality patterns of cancer cases in Brunei. For instance, estimated mortality rates EMR of male for all malignancies by age in 2008 is lowest in Brunei (56.3% versus a highest level of 148.5% in Singapore). Again, EMR of female is third lowest among ASEAN countries (62.8% versus 115.9% in Singapore). Despite this striking low incidence of cancers, Brunei bears the highest mortality rate of female lung carcinomas and the second highest incidence and mortality of colorectal cancer among ASEAN countries [6]. Since 2011, detailed publications began to arise about incidences of certain types of cancer in Brunei, which showed decreases in nasopharyngeal and squamous type esophagus cancers and increases in liver, colorectal, and breast cancers, which will be declared below. Higher life standards and enhanced body mass indices (BMI) may underly some of the associations regarding cancer. In Asian countries, the standardized mortality rates (SMR) and standardized incidence rates (SIR) of ovarian malignancies and their association with the Human Development Index (HDI) were analyzed [9]. The highest SIRs of ovarian malignancies were witnessed in Singapore, Kazakhstan, and Brunei, respectively; and Indonesia, Brunei, and Afghanistan had the highest SMR. While SIR and HDI positively correlated, no correlation was found between the HDI and SMR [9].

Primary hepatic cancer (PHCs) incidence in Brunei between 2000 and 2009 was investigated [10]. Hepatocellular cancer (HCC) and cholangiocarcinoma were the two most common PHCs. A total of 123 cases was determined and the most common type of PHC was HCC (87.8%) followed by cholangiocarcinoma (10.6%). The overall age standardized rate (ASR) of PHC was 8.2/100,000, increasing from 4.5/100,000 population in 2000 to 11.4/100,000 population in 2009 [10]. Chinese had the highest rates (13.1/100,000) compared to the Malays (8.5/100,000) and the indigenous groups [10]. Incidences of esophageal cancer (EC) (squamous cell carcinoma (SCC) and adenocarcinoma (AC)) in Brunei from 1986 to 2012 were determined. The main malignancy was SCC which comprised 89% of ECs. The proportions of SCC among all ECs in the various racial groups were as follows: Indigenous (100%), Chinese (100%), Malays (87.8%), and foreign nationals (20%). Peculiarly, no esophageal AC was found in Chinese and indigenous groups [11]. Nasopharyngeal carcinoma (NPC) cases in Brunei between 1986 to 2014 were analyzed [12]. A total of 450 NPC cases were determined which comprise 4.4% of all malignancy cases during the study period [12]. The most frequent cancer type was the undifferentiated carcinoma (96.4%). The demographic features were mean age 50.4 years old and dominantly Malays 74.4%, followed by Chinese 16.7% [12].

26.3 Early Age Breast Cancers in Southeastern Asia and Brunei

In a study published in 2011, all breast cancer diagnoses registered in the Cancer Registry at RIPAS hospital in Brunei from January 1, 2001, to December 31, 2009, were analyzed [2]. A total of 481 reported cases of breast cancer were identified yet only 200 patients had complete records needed for the different analyses [2]. The incidence of mammary malignancies in Brunei, Malaysia, and Singapore is about half of what was reported in the United States of America [2]. Nonetheless, in Brunei, the age of breast cancer diagnosis was 49.2 years with a peak of incidence occurring in the 45–49 year age group, which is similar to that seen in Malaysia and Singapore, but different from the United States where the median age of disease onset is 61 years [2]. In a larger study again published in 2011, breast cancer patients over a 27-year period (1984–2010) in Brunei were analyzed [3]. The mean age of disease onset was 48.7 years old. More importantly, 22.3% of breast cancers accumulated in the young patient group (less than 40 years old) which is much higher than what has been determined in the Western literature. In the West, breast cancers in the younger group only account for 5% or less [3]. On the other extreme, breast cancers in the elderly (>65 years) only comprised 8.1% of total cases, which is much less than what has been reported in the West [3]. The crude rates of mammary cancer and its proportion among overall cancers in women exerted an increasing trend while patients with malignant phyllodes tumor were much younger compared to the other groups, significantly more so in comparison with the ductal type ($p < 0.05$) [3].

26.4 Other Cancers with Early Age of Onset in Brunei: Emphasis for Colorectal and Neurological Cancers

The incidence and the demographics of young (younger than 45 years) colorectal cancer (CRC) in Brunei Darussalam were assessed. Between 1986 and 2014, there existed 1126 histologically proven CRC which accounted for 15.1% of cancers [13]. Highest percentage of young CRC was encountered among the indigenous (30.8%), followed by the expatriates (29.3%) and Malays (14.3%) and it was lowest among the Chinese (10.8%). A study published in same year revealed that the incidence of young CRC is on the rise in Brunei [14]. Rectal cancers comprised 35.2% ($n = 372$) of all cancers of the colon. For rectal tumors, the ASIRs began to be higher in the 25–29 age group onward whereas for colon malignancies, the increase started from the 45–49 age group [14]. Cancers of the young (any malignancies with a first diagnosis under the age of 40 years) in Brunei between 2000 and 2012 were defined. 18.7% ($n = 1205$) were found as young cancer cases among the 6460 patients diagnosed with cancer [4]. Overall, neurological (54.9%) had the highest proportion of cancers of the young followed by hematological (39.9%), endocrine (38.7%), gynecological/reproductive (30.6%), subcutaneous/dermatological/musculoskeletal (22.3%), and the head and neck region (20.1%) [4]. The incidence was significantly higher among the Malays (20.1%) and expatriates (25.1%) groups in

comparison with the Chinese (10.7%) and indigenous (16.8%) groups ($p < 0.001$ for trend). Here, another peculiar and important feature shall be underlined in regard to brain cancers. Incidences of breast and brain cancers in women are about 2.14- and 1.93-fold greater in Penang than Sarawak in Malaysia, respectively, which are the highest differences among different types of cancers [15]. On the other hand, nasopharyngeal cancer incidence in women is about 1.97-fold greater in Sarawak than Penang indicating that not a general carcinogenic environment would account similarly enhanced risk of breast and brain cancers in Penang [15]. Thus, a genetic tendency may underlie the common higher risk of breast and brain tumors in Penang Malays.

26.5 Thalassemia and Hemoglobinopathies in Southeast Asia and Brunei

α- and β-Thalassemia, Hemoglobin (Hb) Constant Spring, and Hb E are frequently encountered in Southeast Asia [5]. The mutant genes in differing combinations cause more than 60 distinct types of thalassemias, making Southeast Asia the region harboring the most intricate thalassemia genotypes [5]. Nonetheless, less than 20 β-globin gene mutations account constitute more than 90% of β-thalassemia alleles in Southeast Asia and India [16]. In Southeast Asia, the frequency of β-thalassemia can be as high as 10% [17]. The four major thalassemic diseases are homozygous β-thalassemia, β-thalassemia/Hb E, Hb H disease, and Hb Bart's hydrops fetalis (homozygous α-thalassemia) [5]. Compound heterozygosity between Hb E and β-thalassemia leading to β-thalassemia/Hb E disease is common in Bangladesh, Burma, and Sri Lanka [5]. Hb AE Bart's and Hb EF Bart's diseases arise due to gene–gene interactions of α- and β-thalassemias. In Southeast Asia, β-thalassemia mutations are commonly population specific, in which each different ethnic group has its own shared mutants [5]. For instance, thalassemic mutations common in Malays were not found among Chinese and vice versa Chinese mutations were virtually absent in the Malays [18].

In a PCR-based study conducted in Singapore, diagnostic screening was employed on 1116 cord blood samples for neonatal screening [7]. The cord specimens were analyzed in each ethnic group for the most frequent α- and β-globin mutations causing α- and β-thalassemias, respectively [7]. The carrier frequency for α-globin mutations was 6.4% in the Chinese, and 5.2% in Indians, and 4.8% in Malays. The carrier frequency for β-globin mutations was 0.7% in Indians, 2.7% in the Chinese, and 6.3% in Malays [7]. 1000 Brunei patients harboring low levels of Hb, and/or MCV and MCH were investigated for underlying hemoglobinopathies [19]. Of the 1000 subjects analyzed, there were 343 (34.3%) with hemoglobinopathy or thalassemia. β-Thalassemia trait was the predominant disorder accounting for 22.7% of all abnormals [19]. Among 343 patients with thalassemia or hemoglobinopathy, 270 (80.7%) were Malay, 50 (14.6%) were Chinese and 16 (4.7%) were from indigenous tribes [19]. Besides the β-thalassemia trait, there were 4 β-thalassemia major, 37 Hb AE, 5 HbE, and 8 Hb

Eβ cases, leading a total of 281 patients with β-globin abnormalities consisting about 82% of patients with blood indices suggesting likelihood of either thalassemia or hemoglobinopathy [19].

26.6 Malays Have Breast Cancers with Younger Age of Onset and Worse Prognosis than Chinese

Above we indicated that the β-thalassemia mutation carrier frequency was 0.7% in Indians, 2.7% in the Chinese and 6.3% in Malays in Singapore [7]. Bhoo-Pathy evaluated the influence of ethnicity on survival of mammary cancer in Southeast Asia [8]. They reviewed the association between mortality and ethnicity in 5264 patients (Chinese: 71.6%, Malay: 18.4%, Indian: 10.0%) [8]. Malays were diagnosed at younger ages, had bigger tumors and were at later disease stages than the Chinese and Indians. Malays developed more metastasis to axillary lymph nodes at similar tumor volumes and had less differentiated/hormone receptor-negative tumors [8]. Moreover, 5-year survival was longest in the Chinese (75.8%) and shortest in Malays (58.5%) [8]. Malay ethnicity was also associated with higher risk of cause mortality (HR: 1.34), independent of stage, tumoral features, age, and treatment [8]. In an other study conducted on 1034 breast cancer cases revealed that HER2-positive cases and triple-negative cases were more frequent in Malays in comparison with Chinese and natives [20].

Hemoglobinopathies, thalassemias, and their associations with cancer. Oxidative injury, inflammation, and other pathogenetic mechanisms.

For associations between certain malignancies and hemoglobinopathies or thalassemias, the causal pathways seem obvious and illuminated. For instance, enhanced rates of liver cancer in thalassemia major are explained with higher oxidative stress triggered by iron overload [21]. Renal medullary carcinoma (RMC) is a grave cancer mainly encountered in young men suffering from sickle cell disease [22]. Sickle cell disease causes chronic glomerular injury and it is well-established chronic tissue injury-associated inflammation and wound healing responses promote carcinogenesis [23–27]. Such a mechanism may also explain Hb Malmö-associated lung cancers as this hemoglobinopathy (β-97 (FG-4) Histidine→Glutamine) produces a high-affinity hemoglobin variant leading erythrocytosis and lung fibrosis; and lung fibrosis is a result of repetitive alveolar injury which promotes lung carcinogenesis [28, 29]. Nonetheless, there also exist noteworthy observations which indicate that hemoglobin mutations and cancers may associate by mechanisms—at least partially—independent of oxidative injury or inflammation [1]. Sotnikova et al. made the first proposal that cancers and hemoglobin variants may associate through genetic mechanisms on the chromosomal region 11p15.5 [30]. When they studied hemoglobin fractions in 80 patients with Wilms' tumor (nephroblastoma), they witnessed elevated HbF levels in the absence of a thalassemia. Moreover, four children had a uniform abnormal Hb fraction proceeding in front of HbA2 [30]. In one case, this abnormality was encountered in propositus and also in his paternal grandmother and father. Another child with

sporadic Wilms' tumor and his mother was diagnosed to have hereditary persistence of fetal hemoglobin (HPFH). The authors proposed that this phenomenon may associate with the fact that chromosomal loci 11p15.5 includes both β-globin genes and genes predisposing to Wilms tumor [30].

Studies conducted in Italy revealed that in β-thalassemic carriers the titers of medial annual incidence to 10,000 for cancers were shown to be ever higher (46.08 versus 31.49/10,000) [31]. The gastric cancer incidence was higher in β-thalassemia trait than in non-carrier population ($p = 0.02$) [31]. A higher prevalence of thalassemia trait was observed among patients with various malignancies: larynx, esophagus, gallbladder and bile ducts, pancreas, breast, and kidney, yet these differences were not statistically significant [31]. Studies in Thailand revealed important associations between cholangiocarcinoma and hemoglobinopathies. Prevalences of thalassemias and hemoglobinopathies, particularly hemoglobin E, and cholangiocarcinoma were more frequent in the lower part of the Northeast Thailand [32]. Hemoglobin typing in 111 cases of cholangiocarcinoma compared with 146 normal subjects revealed that β-thalassemia trait and hemoglobin E trait were significantly higher in the group with cholangiocarcinoma [32].

Hb Lepore syndrome is generally asymptomatic and is caused an autosomal recessive mutation. Hb Lepore consists of two wild-type α- globin chains (HBA) and two δ-β globin fusion chains and was first identified in Italy. In Italy, Campania is the most affected world area by all Hb Lepore conditions [33]. Noteworthy, 10 malignancies among 161 people (incidence reaching 6%) with heterozygous Hb Lepore were encountered; while the general incidence of malignancies (0.6%) were little higher in β-thalassemia heterozygotes in comparison to normal subjects [33]. Four years later, the same group published their findings based on their experience with 76 families with Hb Lepore, which included cases with 214 heterozygous, 9 homozygous, and 12 combinations with different types of thalassemia [34]. Based on more than 5000 cases of genetic hemoglobinopathies which they followed for 20 years, this group claimed that the cancer risk in the Hb Lepore carriers was 10 times higher than for thalassemics, which is especially prominent for hematological cancers [34].

HB-A2′, also named as HB-B2 or HBA2δ′, is the most frequent δ chain variant of HB-A2, which is occult in clinical terms and laboratory results [35]. HB-B2 is frequent among Africans with the highest incidence in the Herero population belonging to the Bantu-speaking blacks from Namibia [35, 36]. The β-globin genes' loci haplotype linked to the δ-globin variant HB-B2 was defined in Herero's and the high gene frequency of HB-B2 was found to occur by a founder effect [37]. Peculiarly, pediatric central nervous system cancer incidence in the Herero population (26 per million) is prominently higher than the general Namibian population (9.3 per million) [38, 39]. Detailed data of Botswana Hereros revealed that female infants were about three times more likely to survive than males ($p = 0.000001$), and female children were twice as likely to survive than males ($p = 0.01$) [39]. Newborn girls exert higher hypoxic tolerance and exert relatively higher resistance to brain ischemia/hypoxia [40]. Levels of minor adult HB-A2 with the native δ-chain elevate in mountain dwellers and at the period of cardiac ischemia,

afterward declining to normal levels during the recovery period, indicating its potential functions in resistance to hypoxia [41]. The transcription factor inducing δ-chain synthesis, GATA-1 is indued by the female hormone progesterone [41]. Hypothetically, the HBA2 variant HB-B2 caused a survival advantage in female infants of the Herero's via higher protective features of the variant δ-chain. If this different δ-chain provides increased cellular protection, it is also plausible that the survival chance of neuroectodermal cancer stem cells also increase. Alternatively, and probably more likely, the δ-chain gene at 11p15.5 harbors haplotype interactions with neighboring genes involving in glial oncogenesis.

26.7 Genes at 11p15.5 Chromosomal Region Which Associate Both with Early Age Breast Cancers and Neurological Tumors

Loss of heterozygosity (LOH) and epigenetic changes on 11p15.5 in breast and brain tumors: Ali et al. were the first to show LOH in 11p15 region in breast cancer patients [42]. Restriction fragment length polymorphism analyses indicated that the most common sequences losses in mammary cancers resided between the parathyroid hormone and β-globin loci on 11p15.5 [42]. The LOH for chromosome 11 loci significantly associated with tumors lacking hormone receptors (estrogen and progesterone), higher grade disease, and more distant metastases [42]. By transforming human milk epithelial cells with SV40 DNA, an immortalized mammary cell line Hu-MI was obtained, which exhibits the features breast cancer precursor cells [43]. Very noteworthy, a deletion of the 11p15 including the c-Ha-ras and the β-globin genes was revealed in the immortalized cells [43]. Winqvist et al. investigated DNA obtained from 50 matched benign and breast malignant tissues for LOH at loci of the 11p15.5 region [44]. They revealed that 12.5% of patients had LOH at HRAS1, 26.8% at TH (tyrosine hydroxylase), and 33.3% at both DI1S860 and HBB (hemoglobin-β gene cluster) [44]. They suggested that the subregion between HBB and TH is a critical genomic area in mammary carcinogenesis [44]. By analysis of 116 female and 4 male breast cancers, Gudmundsson revealed that LOH at 11pl5.5 was associated with worse prognostic features in breast cancer, including lesser or absent hormone receptors, high S-phase fraction, and lymph node metastasis [45]. Deng and Lichy et al. proposed that LOH at 11p15.5 may be one of the early events in breast carcinogenesis [46, 47].

Karnik et al. investigated 94 matched benign and malignant breast specimens using 17 polymorphic markers that map to 11p15.5–15.4 and determined the residence of a breast cancer suppressor gene between the markers D11S1318 and D11S4088 (~500 kb) within 11p15.5 [48]. LOH at this region was detected in about ~35–45% of breast cancers studied. They also mapped a second region of LOH that spans the markers D11S1338–D11S1323 (~336 kb) at 11p15.5–p15.4, which is lost in ~55–60% of mammary cancers [48]. LOH at region 1 correlated significantly with early stages of cancer; in opposite, the loss of the more proximal region 2, highly correlated with aggressive and metastatic disease [48]. Nakata et al. also defined an

association between the LOH on 11p15.5 and the lack of expression of progesterone receptors, a feature of more aggressive growth [49]. Scelfo et al. proposed a hypomethylating feature on 11p15.5, which specifically occurs on one or more negative regulatory elements, thereby inducing gene silencing and found that such events are encountered in various cancers including breast tumors [50]. Kim et al. investigated microsatellite instability (MSI) at chromosomal region 11p15.5 by microdissection of paraffin-embedded 68 matched benign and malignant breast tissue samples [51]. Intraductal, invasive and metastatic foci in lymph nodes were evaluated for MSI by employing the polymorphic markers D11S922, tyrosine hydroxylase (TH) and D11S988. They found that MSI at D11S922 had a relatively higher frequency than other markers which correlated with breast cancer progression [51]. Han et al. determined that one of the prominent changes in breast cancers which recur under tamoxifen are LOH at 11p15.5–p15.4 [52].

Overall, all these data suggest that 11p15.5, where β-globin gene cluster resides has prominently important associations with breast cancer and particularly with breast malignancies exerting aggressive features. Similar LOH findings were found in regard to high-grade glial tumors. Sonoda et al. investigated 38 gliomas [26 high-grade gliomas (grades III and IV) and 12 low-grade gliomas (grade I and II)] for LOH [53]. LOH was encountered in 8 of 26 high-grade gliomas (31%) but not in the low-grade glial tumors. In the region with LOH, loci on 11p15.5-pter were frequently deleted and they indicated that a potential cancer suppressor gene involved in malignant upgrading of gliomas locates on 11p15.5-pter [53]. Newsham et al. investigated LOH on 11p15 in 24 matched benign tissue and glioma pairs which included anaplastic astrocytoma and glioblastoma [54]. Their findings indicated that a gene involved in the glioma development localizes centromeric in bands 11p15.5–p15.4 [54]. Schiebe et al. analyzed for LOH on 11p15.5 on paired malignant tissues and blood samples from 50 GBM patients. The region 11p15.4–5 was deleted heterozygously in 28% of cases and there was also a significant association of p53 mutations with LOH on 11p15 [55].

HRAS involvement in breast cancers with early age of onset and brain tumors: Some earlier studies suggested that ras mutations are not common (approximately 5%) in breast cancers, yet even these studies underlined that there exists evidence that indicates that Ras pathway involves in breast carcinogenesis [56]. Furthermore, some other studies found more frequent changes in mutation or expression of HRAS in breast cancers. The relative risk attributable to the existence of one HRAS1 allele is moderate, but the combined prevalence of these mutant alleles revealed an important risk of 9.1% of breast cancers [57]. Indeed, recent sensitive studies with allele-specific competitive blocker PCR approach also characterized that HRAS G12D mutation was significantly higher in ductal mammary cancers in comparison with benign breast tissue [58]. More noteworthy observations were made on the involvement of HRAS in breast cancers of young women and in very aggressive triple-negative breast cancers. Peripheral blood DNA analyses on 160 breast cancer patients and on 405 unaffected women in North Carolina revealed that rare HRAS alleles associated with more aggressive tumors particularly in younger women [59]. In parallel, Ozer et al. have found strong expression of HRAS in 13 cases

(37.2%) among 35 breast cancer patients younger than 35 years [60]. Mice with knockout (−/−) of tumor suppressor Ink4a/Arf are susceptible to malignancies such as fibrosarcoma [61]. Kai et al. retrovirally introduced HRAS(G12V) oncogene into Ink4a/Arf (−/−) breast cells in vitro, and inoculated these cells into syngeneic mice. They observed 100% cancer development with tumors negative for hormone receptors and HER2, exerting pathological attributes resembling to triple-negative breast cancer (TNBC) (i.e., central necrosis and pushing borders) in humans [61]. In 2017, analyses on human TNBC confirmed that HRAS was among the major differentially expressed genes in comparison with normal breast tissue [62]. Regarding to high-grade gliomas, there exist conflicting data on HRAS. Some studies showed downregulation of HRAS in human gliomas [63, 64]. On the other hand, some groups have shown overexpression of HRAS in human gliomas and even in correlation with increasing tumor grade [65, 66]. Corroborating these findings, animal experiments have shown that HRAS synergized either with c-myc or hTERT (human telomerase catalytic component) in inducing glial tumors resembling to human glioblastoma [67, 68]. Similar to the situation observed in breast cancer, direct activating mutations of HRAS may be rare in glioblastomas, yet aberrations in regulation of HRAS expression may contribute to tumorigenesis and aggressive behavior.

IGF-2 involvement in breast cancers with early age of onset and brain tumors: Circulatory levels of IGF-1 and IGF-2 correlate positively with risk of carcinogenesis and the IGF system involves in the initiation and progression of essentially all malignant cellular growth [1]. In young people, the P2-4 promoters of IGF-2 are methylated mainly on the suppressed maternal allele, while in aging, this promoter methylation intensifies and comprises the unmethylated allele [69]. Aberrant IGF-2 imprinting in 30% of patients with mammary cancer suggests that aberrant relaxation or pathological loss of IGF-2 imprinting plays an important role in breast oncogenesis [70]. Despite breast cancer incidence is less in African Americans, they arise at earlier age and exert a worse prognosis; and very noteworthy, breast tumor samples from African Americans have increased expression of IGF-2 in comparison with samples obtained from White Americans [71]. Also in triple-negative breast cancers, which occur more frequently in African American patients and in younger subjects, IGF-2 is significantly expressed [72]. A very prominent methylation loss in exon 9 CpG cluster of IGF-2 in mammary cancer tissues was found in comparison with healthy tissue and immunohistochemistry revealed about twofold increase of IGF-2 [73]. The IGF-2 gene is imprinted in the cerebral subcortex in normal conditions, but LOI (loss of imprinting) of IGF-2 gene occurs in ~57% of glial tumors and 2% of anaplastic astrocytomas and 13% of glioblastomas express IGF-2 mRNA levels at intensely increased levels (>50-fold the sample population) [1]. IGF-2 overexpressing tumors often exert loss of PTEN; their proliferative indices are higher and associate with shorter survival [1].

H19/Wilms tumor-2 and its association with aggressivity of breast cancer and glial tumors: The H19 gene residing at 11p15.5 encodes a noncoding RNA strongly synthesized in embryonic development [1]. This oncofetal long noncoding RNA (lncRNA) is expressed at higher levels in breast cancers with bad prognosis,

atypically multidrug-resistant breast cancer cells resistant to adriamycine and to paclitaxel [74–76]. Breast cancer stem cells (BCSCs) synthesize prominent levels of H19, and overexpression of H19 significantly increases colony formation, migratory features, and sphere-forming capabilities of breast cancer cells [77]. In Carolina Breast Cancer Study (1993–2001), analyses of SNPs in 2352 whites and 1447 African Americans revealed that H19 SNPs associated with mammary malignancies in both Whites and African Americans [78]. H19 and its neighboring IGF-2 gene are simultaneously expressed in embryonic mesoderm and endoderm, indicating a shared regulation, and H19 regulates cell proliferation by a cis control on IGF-2 [1]. H19 expression is specifically increased in gliomas of high grade, and its lowering reduces invasion of glioma [1].

SLC22A18 and its putative tumor suppressor functions in breast cancer and gliomas: Solute carrier family 22 member 18 (SLC22A18) gene at 11p15.5 encodes a member of the polyspecific transporters. 1 SLC22A18 displays polymorphic imprinting in adult tissues [79]. Low expression of SLC22A18 associates with shorter survival in breast cancer, indicating a tumor suppressive function of this gene [80]. SLC22A18 is significantly lower expressed in human glial tumors in comparison with normal brain and more so in gliomas with early recurrence following surgery [1]. In 50% of gliomas, SLC22A18 promoter is methylated and absence of the SLC22A18 protein associated with lower survival in glioma patients treated with temozolomide [1].

ILK and its association with aggressive features of breast cancer and gliomas: integrin-linked kinase (ILK) is an ankyrin repeat-containing serine-threonine kinase and could phosphorylate PKB/Akt to stimulate its activity [81, 82]. ILK involves in several stages of carcinogenesis, including blockage of apoptosis, as well as promoting cellular invasion and migration [81]. ILK overexpression in epithelia causes anchorage-independent cellular proliferation and nude mice injection with ILK-overexpressing cells gives rise to tumorigenesis. Enhanced ILK activity correlates with malignant growth, including breast and colon carcinomas and brain tumors [81]. In transgenic mice, breast epithelial-specific overexpression of the ILK causes breast gland hyperplasias and tumors [83]. ILK is an essential mediator of survival of malignant breast cells via the protein kinase B (PKB)/Akt pathway and has a crucial role in the ErbB2-induced breast cancers [84, 85]. ILK also potently inhibits the Hippo tumor suppressor pathway [86]. In glioblastoma, PTEN reduces PKB/Akt phosphorylation via inhibiting ILK signaling and blockage of ILK signaling slowered the growth of PTEN-negative glioblastoma [87]. ILK suppresses E-cadherin via the NF-κB pathway and stimulates glioma cell invasion and migration [88]. ILK also promotes glioblastoma resistance to temozolomide [88].

TSSC3/PHLDA2 and its oncogenic roles in breast cancer and gliomas: TSSC3/PHLDA2 involves in Fas-induced apoptosis and is imprinted in fetal and placental tissues during development [1]. TSSC3/PHLDA2 associates with tumor engraftment in xenografts obtained from breast cancer patients and exerts high expression in triple-negative breast carcinoma with very poor prognosis [89]. PHLDA2/TSSC3 does not undergo imprinting in the healthy adult blood and brain tissues. In opposite, strong allelic bias similar to imprinting is encountered in many glial tumors.

Coexpression of Fas ligand and fas enhances from low- to high-grade glial tumors, and despite this simultaneous expression, glioma cells do not undergo Fas-driven apoptosis [1]. Retention of TSSC3 imprinting in cerebral neoplasias suppresses the Fas apoptotic cascades [1].

p57kip2/CDKN1C and its tumor suppressive functions in breast cancer and gliomas: CDKN1C gene encodes tumor suppressor p57(KIP2) which is a cyclin-dependent kinase (CDK) inhibitor and its family members are mostly suppressed in human malignancies via DNA methylation [90]. CDKN1C expression in breast cancers is repressed and mammary cancers with low CDKN1C levels associate with shorter survival [90]. Chinese breast cancer patients with no p57KIP2 expression exerted significantly higher metastasis [91]. Associations between 35 mammary malignancy-susceptibility gene loci and the overall survival (OS) in 10,255 breast cancer subjects from the National Cancer Institute Breast and Prostate Cancer Cohort Consortium were studied. rs3817198 SNP was correlated with better OS which associated with higher levels of CDKN1C [92]. In glioma, induction of CDKN1C/p57KIP2 blocks cell proliferation and induces cellular senescence and also inhibits motility and invasion [93, 94]. CDKN1C and miR-25 inversely associate in gliomas; miR-25 increases cell proliferation, vice versa silencing of endogenous miR-25 rescues CDKN1C expression and decreases glioma cell growth [95]. In parallel, copy number gains of CDKN1C correlate with longer survival of glioblastoma patients [96].

26.8 Conclusions

Breast cancers and high-grade glial brain tumors are devastating public health problems due to their frequency and fatality, respectively. As suggested above, Southeast Asian countries have very high incidences of β-hemoglobinopathies and thalassemias [5, 19]. A very noteworthy and paradoxical issue is that these countries have a lower incidence of breast cancer despite with early age of onset in comparison with Western countries [14]. There are insufficient data on the age distribution of neurological cancers in Southeast Asian countries in general, yet among the different organ systems effected, neurological cancers had the biggest proportion (54.9%) of young cancers (age less than 40 years) in Brunei [4]. Among ASEAN countries, Brunei has the highest GDP per capita and its small population is mostly composed by Malays (66%) [2]. In a study conducted in Singapore on 1116 cord blood samples for neonatal screening, β-thalassemia mutations were highest in Malays (6.3%) versus 2.7 % in Chines and 0.7% in Indians [7]. Above we indicated that in Southeast Asia, β-thalassemia mutations exert ethnicity-associated distribution; such as thalassemic mutations common in Malays were not found among Chinese and vice versa [18]. Importantly, Malays with breast cancer were diagnosed at a significantly younger age, with bigger and hormone receptor-negative tumors, and at later stages than Indians and Chinese [8].

Breast cancer is a prominently heterogeneous malignancy in which even common pathologic and clinical features associate with distinct outcomes [97]. Hence, staging

systems based on clinicopathologic features reached their usefulness limit which impelled the necessity for additional molecular biomarkers to predict patients' outcome and treatment [97]. Besides TNM staging, histopathological grade, estrogen and progesterone receptors and HER2 expression, screening for epigenetic and genetic changes in 11p15.5 gene loci encompassing β-globin genes and critical tumor suppressor genes may provide novel clues to predict prognosis and to design novel treatment strategies. One major obstacle of our hypothesis is the lack of early age breast and neurological cancers in Middle East, Mediterranean, and African countries, where thalassemias and hemoglobinopathies are also prevalent. This difference may be explained with regional differences of mutation types and differential splicing of immunomodulatory hemoglobin peptides which are called as hemorphins and with the fact that HLA genotype may exert race-dependent differences which would modify risk of cancer [1].

Here, we propose that the hematological diseases which develop due to β-globin gene mutations, i.e., thalassemias and hemoglobinopathies and early age breast cancers and gliomas may have at least partially shared pathological etiology. β-Globin cluster and tumor susceptibility genes residing on 11p15.5 chromosomal region may exert haplotypal associations or hemoglobin mutations may cause sustained oxidative stress and inflammation to promote tumorigenesis. Proval of this hypothesis may pave to develop many novel and efficient ways of cancer prediction and prevention. Simple electrophoresis or HPLC tests in microcytic anemia patients may help to detect carriers of hemoglobinopathies and these subjects may be screened for cancer in a more detailed and/or frequent manner.

Conflict of Interest None to declare

References

1. Altinoz MA, Elmaci I, Ince B, Ozpinar A, Sav AM (2016) Hemoglobins, hemorphins, and 11p15.5 chromosomal region in cancer biology and immunity with special emphasis for brain tumors. J Neurol Surg A Cent Eur Neurosurg 77:247–257
2. Tan S, Abdullah MS, Telisinghe PU, Ramasamy R (2011) Breast cancer in Brunei Darussalam-Incidence and the role of evaluation of molecular markers. Brunei Int Med J 7:250–259
3. Chong VH, Telisinghe PU (2011) Rising incidence of breast cancer in Brunei Darussalam. Brunei Int Med J 7:260–268
4. Mohammad IA, Bujang MR, Telisinghe PU, Abdullah MS, Chong CF, Chong VH (2014) Cancers of the young population in Brunei Darussalam. Asian Pac J Cancer Prev 15:6357–6362
5. Fucharoen S, Winichagoon P (2011) Haemoglobinopathies in southeast Asia. Indian J Med Res 134:498–506
6. Kimman M, Norman R, Jan S, Kingston D, Woodward M (2012) The burden of cancer in member countries of the Association of Southeast Asian Nations (ASEAN). Asian Pac J Cancer Prev 13:411–420
7. Kham SK, Quah TC, Loong AM et al (2004) A molecular epidemiologic study of thalassemia using newborns' cord blood in a multiracial Asian population in Singapore: results and recommendations for a population screening program. J Pediatr Hematol Oncol 26:817–819
8. Bhoo-Pathy N, Hartman M, Yip CH et al (2012) Ethnic differences in survival after breast cancer in South East Asia. PLoS One 7:e30995. https://doi.org/10.1371/journal.pone.0030995

9. Razi S, Ghoncheh M, Mohammadian-Hafshejani A, Aziznejhad H, Mohammadian M, Salehiniya H (2016) The incidence and mortality of ovarian cancer and their relationship with the Human Development Index in Asia. Ecancermedicalscience 10:628. https://doi.org/10.3332/ecancer.2016.628
10. Chong RJ, Abdullah MS, Hossain MM, Telisinghe PU, Chong VH (2013) Rising incidence of primary liver cancer in Brunei Darussalam. Asian Pac J Cancer Prev 14:3473–3477
11. Chong VH, Telisinghe PU, Chong CF (2015) Esophageal cancer in Brunei Darussalam over a three decade period: an epidemiologic study of trends and differences between genders and racial groups. Asian Pac J Cancer Prev 16:4123–4126
12. Chong VH, Telisinghe PU, Lim E, Abdullah MS, Idris F, Chong CF (2015) Declining incidence of nasopharyngeal carcinoma in Brunei Darussalam: a three decade study (1986–2014). Asian Pac J Cancer Prev 16:709
13. Koh KS, Telisinghe PU, Bickle I, Abdullah MS, Chong CF, Chong VH (2015) Characteristics of young colorectal cancer in Brunei Darussalam: an epidemiologic study of 29 years (1986–2014). Asian Pac J Cancer Prev 16:3279–3283
14. Chong VH, Telisinghe PU, Bickle I, Abdullah MS, Lim E, Chong CF (2015) Increasing incidence of colorectal cancer, starting at a younger age for rectal compared to colon cancer in Brunei Darussalam. Asian Pac J Cancer Prev 16:5063–5067
15. Moore MA, Manan AA, Chow KY et al (2010) Cancer epidemiology and control in peninsular and island South-East Asia—past, present and future. Asian Pac J Cancer Prev 11(Suppl 2):81–98
16. Wang W, Kham SK, Yeo GH, Quah TC, Chong SS (2003) Multiplex minisequencing screen for common Southeast Asian and Indian beta-thalassemia mutations. Clin Chem 49:209–218
17. Pramoonjago P, Harahap A, Taufani RA, Setianingsih I, Marzuki S, Harahap A (1999) Rapid screening for the most common beta thalassaemia mutations in south east Asia by PCR based restriction fragment length polymorphism analysis (PCR-RFLP). J Med Genet 36:937–938
18. George E, Li HJ, Fei YJ et al (1992) Types of thalassemia among patients attending a large university clinic in Kuala Lumpur, Malaysia. Hemoglobin 16:51–66
19. Ismail JB (1992) Thalassaemia and haemoglobinopathies in Brunei Darussalam. Med J Malaysia 47:98–102
20. Devi CR, Tang TS, Corbex M (2012) Incidence and risk factors for breast cancer subtypes in three distinct South-East Asian ethnic groups: Chinese, Malay and natives of Sarawak. Malaysia Int J Cancer 131:2869–2877
21. Moukhadder HM, Halawi R, Cappellini MD, Taher AT (2017) Hepatocellular carcinoma as an emerging morbidity in the thalassemia syndromes: a comprehensive review. Cancer 123:751–758
22. Hakimi AA, Koi PT, Milhoua PM et al (2007) Renal medullary carcinoma: the Bronx experience. Urology 70:878–882
23. Nath KA, Hebbel RP (2015) Sickle cell disease: renal manifestations and mechanisms. Nat Rev Nephrol 11:161–171
24. Kluwe J, Mencin A, Schwabe RF (2009) Toll-like receptors, wound healing, and carcinogenesis. J Mol Med (Berl) 87:125–138
25. Gonda TA, Tu S, Wang TC (2009) Chronic inflammation, the tumor microenvironment and carcinogenesis. Cell Cycle 8:2005–2013
26. Antsiferova M, Werner S (2012) The bright and the dark sides of activin in wound healing and cancer. J Cell Sci 125(Pt 17):3929–3937. https://doi.org/10.1242/jcs.094789
27. Brockmann L, Giannou AD, Gagliani N, Huber S (2017) Regulation of T(H)17 cells and associated cytokines in wound healing, tissue regeneration, and carcinogenesis. Int J Mol Sci 18(5):pii: E1033. https://doi.org/10.3390/ijms18051033
28. Berglund S, Linell F (1972) Fibrosis and carcinoma of the lung in a family with haemoglobin Malmö. Anatomic findings. Scand J Haematol 9:424–432

29. Chambers RC, Mercer PF (2015) Mechanisms of alveolar epithelial injury, repair, and fibrosis. Ann Am Thorac Soc 12(Suppl 1):S16–S20. https://doi.org/10.1513/AnnalsATS.201410-448MG
30. Sotnikova EN, Durnov LA, Zhordania RV, Gar'kavtseva RF, Bukhny AF (1984) [Changes in the hemoglobin fractions of nephroblastoma patients]. Genetika 20:357–361
31. Gallerani M, Pulga A, Martinelli L et al (1990) Heterozygote beta-thalassemia and neoplastic pathology. Minerva Med 81:175–180
32. Insiripong S, Thaisamakr S, Amatachaya C (1997) Hemoglobin typing in cholangiocarcinoma. Southeast Asian J Trop Med Public Health 28:424–427
33. Quattrin N, Mastrobouni A, Laudisio FL, Brancaccio V, Pagnini D (1976) Hb Lepore and (haemo-) blastomata. Folia Haematol Int Mag Klin Morphol Blutforsch 103:915–919
34. Quattrin N, Luzzatto L, Quattrin S Jr (1980) New clinical and biochemical findings from 235 patients with hemoglobin Lepore. Ann N Y Acad Sci 344:364–374
35. Nusrat M, Moiz B, Nasir A, Rasool HM (2011) An insight into the suspected HbA2′ cases detected by high performance liquid chromatography in Pakistan. BMC Res Notes 4:103
36. Bennani M, Mombo LE, Chaventre A et al (2003) Origin of Hb A2′ (Hb B2) [delta16(A13)Gly —> Arg (GGC —> CGC)]. [delta16(A13)Gly–> Arg (GGC–> CGC)]. Hemoglobin 27:105–110
37. Spurdle AB, Krause A, Ramsay M, Jenkins T (1994) The high frequency of the Hb B2 variant in the Herero population: a founder effect? Hemoglobin 18:317–323
38. Wessels G, Hesseling PB (1996) Unusual distribution of childhood cancer in Namibia. Pediatr Hematol Oncol 13:9–20
39. Harpending HC, Pennington R (1991) Age structure and sex-biased mortality among Herero pastoralists. Hum Biol 63:329–353
40. KlymenkoTM. (1999) Accounting for sexual dimorphism in neonatology [in Ukrainian]. Lik Sprava 6:73–75
41. İnce B, Guloksuz S, Altınbaş K, Oral ET, Alpkan LR, Altinoz MA (2013) Minor hemoglobins HbA2 and HbF associate with disease severity in bipolar disorder with a likely protective role of HbA2 against post-partum episodes. J Affect Disord 151:405–408
42. Ali IU, Lidereau R, Theillet C, Callahan R (1987) Reduction to homozygosity of genes on chromosome 11 in human breast neoplasia. Science 238:185–188
43. Garcia I, Brandt D, Weintraub J, Zhou WG, Aapro M (1991) Loss of heterozygosity for the short arm of chromosome 11 (11p15) in human milk epithelial cells immortalized by microinjection of SV40 DNA. Cancer Res 51:294–300
44. Winqvist R, Mannermaa A, Alavaikko M et al (1993) Refinement of regional loss of heterozygosity for chromosome 11p15.5 in human breast tumors. Cancer Res 53:4486–4488
45. Gudmundsson J, Barkardottir RB, Eiriksdottir G et al (1995) Loss of heterozygosity at chromosome 11 in breast cancer: association of prognostic factors with genetic alterations. Br J Cancer 72:696–701
46. Deng G, Lu Y, Zlotnikov G, Thor AD, Smith HS (1996) Loss of heterozygosity in normal tissue adjacent to breast carcinomas. Science 274:2057–2059
47. Lichy JH, Zavar M, Tsai MM, O'Leary TJ, Taubenberger JK (1998) Loss of heterozygosity on chromosome 11p15 during histological progression in microdissected ductal carcinoma of the breast. Am J Pathol 153:271–278
48. Karnik P, Paris M, Williams BR, Casey G, Crowe J, Chen P (1998) Two distinct tumor suppressor loci within chromosome 11p15 implicated in breast cancer progression and metastasis. Hum Mol Genet 7:895–903
49. Nakata T, Yoshimoto M, Kasumi F, Akiyama F, Sakamoto G, Nakamura Y et al (1998) Identification of a new commonly deleted region within a 2-cM interval of chromosome 11p11 in breast cancers. Eur J Cancer 34:417–421
50. Scelfo RA, Schwienbacher C, Veronese A et al (2002) Loss of methylation at chromosome 11p15.5 is common in human adult tumors. Oncogene 21:2564–2572

51. Kim DJ, Park JY, Lee MH, Sohn YK (2004) The role of microsatellite instability at chromosome 11p15.5 in the progression of breast ductal carcinoma. J Korean Med Sci 19:698–703
52. Han W, Han MR, Kang JJ et al (2006) Genomic alterations identified by array comparative genomic hybridization as prognostic markers in tamoxifen-treated estrogen receptor-positive breast cancer. BMC Cancer 6:92. PubMed PMID: 16608533
53. Sonoda Y, Iizuka M, Yasuda J et al (1995) Loss of heterozygosity at 11p15 in malignant glioma. Cancer Res 55:2166–2168
54. Newsham IF, Gorse KM, Rempel SA, Luckey J, Golden JB, Bögler O (2000) Use of horizontal ultrathin gel electrophoresis to analyze allelic deletions in chromosome band 11p15.5 in gliomas. Neuro-Oncology 2:1–5
55. Schiebe M, Ohneseit P, Hoffmann W, Meyermann R, Rodemann HP, Bamberg M (2001) Loss of heterozygosity at 11p15 and p53 alterations in malignant gliomas. J Cancer Res Clin Oncol 127:325–328
56. Clark GJ, Der CJ (1995) Aberrant function of the Ras signal transduction pathway in human breast cancer. Breast Cancer Res Treat 35:133–144
57. Krontiris TG, Devlin B, Karp DD, Robert NJ, Risch N (1993) An association between the risk of cancer and mutations in the HRAS1 minisatellite locus. N Engl J Med 329:517–523
58. Myers MB, Banda M, McKim KL, Wang Y, Powell MJ, Parsons BL (2016) Breast cancer heterogeneity examined by high-sensitivity quantification of PIK3CA, KRAS, HRAS, and BRAF mutations in normal breast and ductal carcinomas. Neoplasia 18:253–263
59. Garrett PA, Hulka BS, Kim YL, Farber RA (1993) HRAS protooncogene polymorphism and breast cancer. Cancer Epidemiol Biomark Prev 2(2):131–138
60. Ozer E, Sis B, Ozen E, Sakizli M, Canda T, Sarioğlu S (2000) BRCA1, C-erbB-2, and H-ras gene expressions in young women with breast cancer. An immunohistochemical study. Appl Immunohistochem Mol Morphol 8(1):12–18
61. Kai K, Iwamoto T, Kobayashi T et al (2014) Ink4a/Arf(−/−) and HRAS(G12V) transform mouse mammary cells into triple-negative breast cancer containing tumorigenic CD49f(-) quiescent cells. Oncogene 33:440–448
62. Peng C, Ma W, Xia W, Zheng W (2017) Integrated analysis of differentially expressed genes and pathways in triple-negative breast cancer. Mol Med Rep 15:1087–1094
63. Blumenstock M, Prosenc N, Patt S, Pfanne K, Drum F, Cervos-Navarro J (1991) In contrast to EGFr gene overexpression, H-ras gene expression decreases in human gliomas. Anticancer Res 11:1353–1357
64. Patt S, Cervós-Navarro J (1992) Combined erbB gene overexpression and decreased H-ras gene expression in human gliomas. Acta Histochem Suppl 42:131–138
65. Arvanitis D, Malliri A, Antoniou D, Linardopoulos S, Field JK, Spandidos DA (1991) Ras p21 expression in brain tumors: elevated expression in malignant astrocytomas and glioblastomas multiforme. In Vivo 5:317–321
66. Orian JM, Vasilopoulos K, Yoshida S, Kaye AH, Chow CW, Gonzales MF (1992) Overexpression of multiple oncogenes related to histological grade of astrocytic glioma. Br J Cancer 66:106–112
67. Barnett SC, Robertson L, Graham D, Allan D, Rampling R (1998) Oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells transformed with c-myc and H-ras form high-grade glioma after stereotactic injection into the rat brain. Carcinogenesis 19:1529–1537
68. Sonoda Y, Ozawa T, Hirose Y et al (2001) Formation of intracranial tumors by genetically modified human astrocytes defines four pathways critical in the development of human anaplastic astrocytoma. Cancer Res 61:4956–4960
69. Issa JP, Vertino PM, Boehm CD, Newsham IF, Baylin SB (1996) Switch from monoallelic to biallelic human IGF2 promoter methylation during aging and carcinogenesis. Proc Natl Acad Sci U S A 93:11757–11762
70. Wu HK, Squire JA, Catzavelos CG, Weksberg R (1997) Relaxation of imprinting of human insulin-like growth factor II gene, IGF2, in sporadic breast carcinomas. Biochem Biophys Res Commun 235:123–129

71. Kalla Singh S, Tan QW, Brito C, De León M, Garberoglio C, De León D (2010) Differential insulin-like growth factor II (IGF-II) expression: a potential role for breast cancer survival disparity. Growth Hormon IGF Res 20:162–170
72. Hamilton N, Márquez-Garbán D, Mah V et al (2015) Biologic roles of estrogen receptor-β and insulin-like growth factor-2 in triple-negative breast cancer. Biomed Res Int 2015:925703. https://doi.org/10.1155/2015/925703
73. Shetty PJ, Movva S, Pasupuleti N et al (2011) Regulation of IGF2 transcript and protein expression by altered methylation in breast cancer. J Cancer Res Clin Oncol 137:339–345
74. Dugimont T, Curgy JJ, Wernert N et al (1995) The H19 gene is expressed within both epithelial and stromal components of human invasive adenocarcinomas. Biol Cell 85:117–124
75. Doyle LA, Yang W, Rishi AK, Gao Y, Ross DD (1996) H19 gene overexpression in atypical multidrug-resistant cells associated with expression of a 95-kilodalton membrane glycoprotein. Cancer Res 56:2904–2907
76. Si X, Zang R, Zhang E et al (2016) LncRNA H19 confers chemoresistance in ERα-positive breast cancer through epigenetic silencing of the pro-apoptotic gene BIK. Oncotarget 7:81452–81462
77. Peng F, Li TT, Wang KL et al (2017) H19/let-7/LIN28 reciprocal negative regulatory circuit promotes breast cancer stem cell maintenance. Cell Death Dis 8:e2569. https://doi.org/10.1038/cddis.2016.438
78. O'Brien KM, Cole SR, Poole C et al (2014) Replication of breast cancer susceptibility loci in whites and African Americans using a Bayesian approach. Am J Epidemiol 179:382–394
79. Gallagher E, Mc Goldrick A, Chung WY et al (2006) Gain of imprinting of SLC22A18 sense and antisense transcripts in human breast cancer. Genomics 88:12–17
80. He H, Xu C, Zhao Z, Qin X, Xu H, Zhang H (2011) Low expression of SLC22A18 predicts poor survival outcome in patients with breast cancer after surgery. Cancer Epidemiol 35:279–285
81. Yoganathan N, Yee A, Zhang Z et al (2002) Integrin-linked kinase, a promising cancer therapeutic target: biochemical and biological properties. Pharmacol Ther 93:233–242
82. Attwell S, Roskelley C, Dedhar S (2000) The integrin-linked kinase (ILK) suppresses anoikis. Oncogene 19:3811–3815
83. White DE, Cardiff RD, Dedhar S, Muller WJ (2001) Mammary epithelial-specific expression of the integrin-linked kinase (ILK) results in the induction of mammary gland hyperplasias and tumors in transgenic mice. Oncogene 20:7064–7072
84. Troussard AA, McDonald PC, Wederell ED et al (2006) Preferential dependence of breast cancer cells versus normal cells on integrin-linked kinase for protein kinase B/Akt activation and cell survival. Cancer Res 66:393–403
85. Pontier SM, Huck L, White DE et al (2010) Integrin-linked kinase has a critical role in ErbB2 mammary tumor progression: implications for human breast cancer. Oncogene 29:3374–3385
86. Serrano I, McDonald PC, Lock F, Muller WJ, Dedhar S (2013) Inactivation of the Hippo tumour suppressor pathway by integrin-linked kinase. Nat Commun 4:2976. https://doi.org/10.1038/ncomms3976
87. Obara S, Nakata M, Takeshima H et al (2004) Integrin-linked kinase (ILK) regulation of the cell viability in PTEN mutant glioblastoma and in vitro inhibition by the specific COX-2 inhibitor NS-398. Cancer Lett 208:115–122
88. Liang F, Wang B, Bao L, Zhao YS, Zhang SM, Zhang SQ (2017) Overexpression of ILK promotes temozolomide resistance in glioma cells. Mol Med Rep 15:1297–1304
89. Moon HG, Oh K, Lee J, Lee M, Kim JY, Yoo TK et al (2015) Prognostic and functional importance of the engraftment-associated genes in the patient-derived xenograft models of triple-negative breast cancers. Breast Cancer Res Treat 154:13–22
90. Yang X, Karuturi RK, Sun F, Aau M, Yu K, Shao R et al (2009) CDKN1C (p57) is a direct target of EZH2 and suppressed by multiple epigenetic mechanisms in breast cancer cells. PLoS One 4:e5011. https://doi.org/10.1371/journal.pone.0005011

91. Xu XY, Wang WQ, Zhang L, Li YM, Tang M, Jiang N et al (2012) Clinical implications of p57 KIP2 expression in breast cancer. Asian Pac J Cancer Prev 13:5033–5036
92. Barrdahl M, Canzian F, Lindström S et al (2015) Association of breast cancer risk loci with breast cancer survival. Int J Cancer 137:2837–2845
93. Tsugu A, Sakai K, Dirks PB et al (2000) Expression of p57(KIP2) potently blocks the growth of human astrocytomas and induces cell senescence. Am J Pathol 157:919–932
94. Sakai K, Peraud A, Mainprize T et al (2004) Inducible expression of p57KIP2 inhibits glioma cell motility and invasion. J Neuro-Oncol 68:217–223
95. Zhang J, Gong X, Tian K et al (2015) miR-25 promotes glioma cell proliferation by targeting CDKN1C. Biomed Pharmacother 71:7–14
96. Nakahara Y, Shiraishi T, Okamoto H et al (2004) Detrended fluctuation analysis of genome-wide copy number profiles of glioblastomas using array-based comparative genomic hybridization. Neuro-Oncology 6:281–289
97. Corso G, Figueiredo J, De Angelis SP, Corso F, Girardi A, Pereira J, Seruca R, Bonanni B, Carneiro P, Pravettoni G, Guerini Rocco E, Veronesi P, Montagna G, Sacchini V, Gandini S (2020) E-cadherin deregulation in breast cancer. J Cell Mol Med 24(11):5930–5936. https://doi.org/10.1111/jcmm.15140