David Malkin *Editor*

The Hereditary Basis of Childhood Cancer

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Editor
David Malkin
University of Toronto
Departments of Pediatrics and Medical Biophysics
The Hospital for Sick Children
Division of Hematology/Oncology
Toronto, ON, Canada

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Dedicated to the Memory of

Professor Thierry Frebourg

Physician, scientist, scholar, colleague, mentor, and great friend.

Preface

Cancer is the most common cause of disease-related death in children beyond the newborn period. In the absence of long-term exposure to exogenous factors that are commonly associated with adult-onset cancers, the role of genetic risk factors has been considered, at least conceptually, to play a more significant role in the etiology of childhood onset cancers. However, even the most comprehensive genome-wide sequencing efforts across multiple disease types has only revealed about 15% of children to harbor a germline pathogenic or likely pathogenic variant that their cancer can be attributed to. This frequency does vary widely depending on the particular cancer under question, as well as the depth and breadth of sequencing. Furthermore, this approach does not consider evolving technologies that are exploring alterations in non-coding regions, as well as emerging questions of the effect of polygenic risk. Nonetheless, it is clear that we now live in an exciting era of early twenty-first century medicine that applies new 'omics tools to cancer in general and pediatric cancer in particular, creates new platforms and devices to facilitate identification of risk prediction, educates patients, families, and health care practitioners about the importance of family history (and personal cancer history) in determining genetic risk, and integrates multi-disciplinary fields to the study of cancer genetics.

With this explosion of tools at our disposal to resolve the questions of the hereditary basis of childhood cancer more clearly, we envisioned an opportunity to develop a textbook that explores the field in more detail. This book was not intended to simply provide a descriptive listing of various cancer predisposition syndromes and the various genotype–phenotype relationships. This has been done very effectively in many other textbooks. Rather, it is meant to provide the reader with an overview of the breadth of issues including the roles of genetic counseling (Chap. 14), integration of artificial intelligence (Chap. 16), psychosocial context (Chap. 15), and ethical frameworks (Chap. 13) on which the evaluation of these patients and their families and the strategies for implementation of testing and surveillance need to be considered. Furthermore, as access to many of the tools and technical platforms are severely limited in under-resourced countries around the world, an examination of how to adapt their use is warranted and discussed (Chap. 12).

The first several chapters of the textbook are divided into discussions of either individual hereditary cancer syndromes (Chaps. 1, 4, 5, 7, and 9) or diseases (Chaps. 3, 6, and 11) or clusters of systems-based diseases (Chaps. 2, 8, and 10). These are then followed by chapters as noted above that explore the medical and societal contexts in which these diseases are studied, evaluated, and medically managed. There is, necessarily, some overlap between certain chapters as the principles discussed frequently cross multiple clinical settings. At the same time, the style and content within each of the "disease-associated" chapters reflect the unique perspectives of the authors. As such, the presentation of concepts is written in a non-formulaic manner from chapter to chapter.

Ever since the pioneering work of Drs. Alfred Knudson, Louise Strong, Joseph Fraumeni, Frederick Li, and many others established the principles on which we now understand the importance of the genetic basis of cancer, the integration initially of gene-based sequencing, panel sequencing, and more recently next-generation sequencing has raised as many intriguing questions about genetic risk as it has provided answers. There can be no doubt that our understanding of the field will continue to rapidly evolve, which will lead to ever more effective approaches to the medical and psychosocial management of our patients. The genetic basis of human cancer has always been deeply rooted in discoveries made in the "rare" pediatric cancer setting. As such, this textbook is timely in summarizing the many important lessons that have been learned—and offering some insight into directions that new research questions will take us.

I take this opportunity to thank all the authors for their dedication to their work and for sharing their insights to create this textbook. The breadth of expertise that has been gathered here, representing an international scope of practice and scientific thought, is truly remarkable. I am sure that as soon as this book hits the shelves, we will be engaged in updating it for another edition. For even now as this Preface is written, new discoveries continue to emerge, practice guidelines change, and the future continues to evolve.

Toronto, ON, Canada David Malkin
July 2021

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About the Editor

David Malkin is Professor of Pediatrics and Medical Biophysics in the Faculty of Medicine, University of Toronto. He holds the CIBC Children's Foundation Chair in Child Health Research, is a Senior Staff Oncologist in the Division of Hematology/Oncology, Director of the Cancer Genetics program, and a Senior Scientist in the Genetics and Genome Biology Program at The Hospital for Sick Children in Toronto. Dr. Malkin is Co-Lead of the SickKids Precision Child Health initiative. He is co-Director of the SickKids Cancer Sequencing (KiCS) program which integrates and translates next-generation sequencing into clinical care of children with cancer, and Director of the pan-Canadian multi-institutional PRecision Oncology For Young peopLE (PROFYLE) initiative which is establishing a pipeline to incorporate next-generation sequencing into novel clinical trials ("precision oncology") for children and young adults with hard-to-cure cancers across Canada. Dr. Malkin's research program focuses on genetic and genomic mechanisms of childhood cancer susceptibility which he has explored particularly in the context of *TP53* and Li-Fraumeni syndrome. Recently, his work has addressed the application of genomics to develop rational clinical surveillance and treatment guidelines for children and adults at genetic "high risk" for cancer. He has published over 250 peer-reviewed articles and has received several awards recognizing his dedication to clinical care, advocacy, research, medical education, and mentorship.

Chapter 1
Li-Fraumeni Syndrome

Anita Villani, Thierry Frebourg, and David Malkin

Abstract Li-Fraumeni syndrome (LFS) is a prototypic cancer susceptibility syndrome, resulting from germline pathogenic variants in the tumor suppressor gene, *TP53*. Originally described in 1969 (Li, Fraumeni, Jr. Annals of Internal Medicine. 71:747–752) as a familial syndrome characterized by soft tissue sarcoma, breast cancer, and other neoplasms in children and young adults, work over the ensuing decades has led to the recognition of an expanded phenotype of early-onset cancers with varying degrees of aggressiveness. The marked clinical heterogeneity in site and age of cancer onset represents one of the challenges inherent in managing patients with this syndrome. Advances in our understanding of the genomic basis of LFS will play an important role in refining genotype-phenotype correlations within and between LFS families. Furthermore, it can also be expected that the role of p53 in human cancer generally will be more clearly articulated through the ongoing study of the progression to cancer in these patients. This chapter will summarize the current state of the art in the study and genetics-based management of LFS.

Keywords Li-Fraumeni syndrome · p53 · Surveillance · Sarcoma · Brain tumor · Breast cancer · Adrenocortical cancer

1.1 Epidemiology/Tumor Spectrum

A number of epidemiological studies have estimated the remarkable lifetime cancer risk observed in individuals with LFS [1]. In a study of 214 LFS families that identified 415 mutation carriers, 322 (78%) developed tumors and 43% had developed multiple malignancies [2]. A significant number of cancers were diagnosed at a young age: 4% during the first year of life, 22% by 5 years, and 41% by 18 years of age. In children and adolescents, the LFS tumor spectrum was characterized by osteosarcomas

A. Villani · D. Malkin (✉)
Hematology/Oncology, The Hospital for Sick Children, Toronto, ON, Canada
e-mail: David.malkin@sickkids.ca

T. Frebourg (Deceased)

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(30%), adrenocortical carcinoma (27%), central nervous system (CNS) tumors (26%), and soft tissue sarcomas (23%). Breast cancer was the most frequent cancer observed (79% of females) followed by soft tissue sarcomas in 27% of adults.

Investigators at the NCI (Bethesda, Maryland) evaluated 107 families with 286 *TP53* mutation carriers [3]. The cumulative cancer incidence was 50% by age 31 years for females and 46 years for males. By age 70, cancer penetrance was nearly 100% for both sexes, and earlier onset in females was largely attributed to breast cancer. Among females, the cumulative incidence of breast cancer, soft tissue sarcoma, brain cancer, and osteosarcoma was 54%, 15%, 6%, and 5% by age 70, respectively. Among males, the incidences of soft tissue sarcoma, brain cancer, and osteosarcoma were 22%, 19%, and 11%, respectively. Notably, ascertainment bias is likely to cause an overestimation of tumor risk in LFS, as most analyses are performed with affected children having familial history of cancer or multiple primaries. Therefore, it is likely that the global penetrance of germline *TP53* alterations has been overestimated. As *TP53* testing becomes increasingly widespread in cancer patients, germline carriers are now more frequently identified in patients and families with adult-onset cancers [4]. Importantly, a pattern of genetic anticipation is often observed in individual LFS families, although the molecular mechanism underlying this phenomenon remains unclear [5].

"Classic" LFS component tumors have traditionally been defined as soft tissue sarcomas, osteosarcomas, premenopausal breast cancer, brain tumors, leukemias, and adrenocortical carcinoma. This tumor spectrum has not only been expanded [6–8] (Fig. 1.1) but also further refined, by a number of epidemiological studies, and

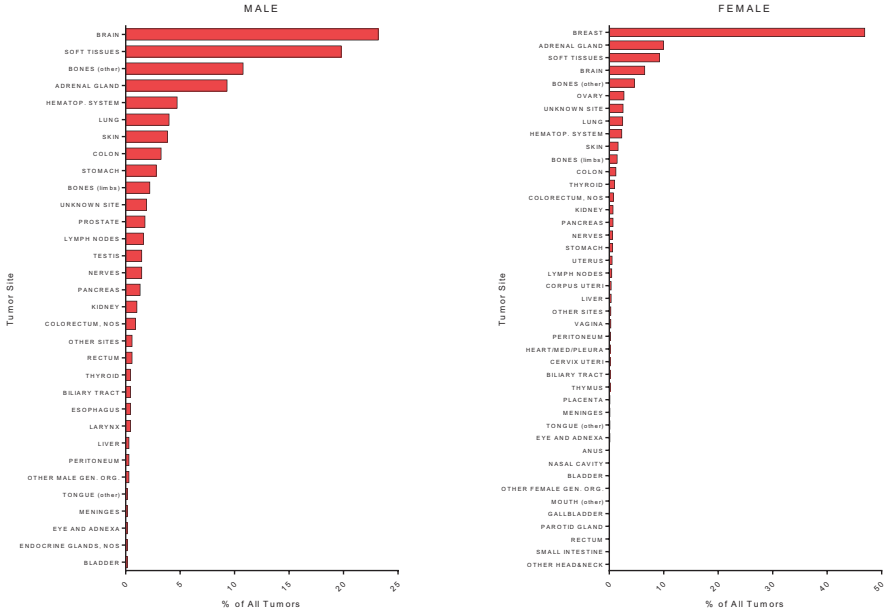

Fig. 1.1 Tumor distributions for male ($n = 677$) and female ($n = 1738$) carriers of germline *TP53* mutations. Source [37]

important lessons have emerged. Firstly, common carcinomas in the general population, including the lung, colon, cervix, ovary, and prostate, are seen infrequently in carriers of a germline *TP53* mutation [8, 9]. Their occurrence, however, is characterized by a much earlier age of onset compared to sporadic tumors in the general population – up to two to three decades sooner [8–11]. This "phenotypic switch" may be a reflection of the differential effects of a late mutational event in *TP53*, as commonly occurs in sporadic lung and colon cancer, as opposed to an "early event" in oncogenesis in individuals with germline *TP53* mutations. The second lesson, which continues to develop, is that certain specific histologic and molecular subtypes of classic LFS component tumors appear to be enriched in the LFS population. Among CNS tumors, 50–100% of choroid plexus carcinomas have been shown to be associated with germline *TP53* mutations and have been incorporated into more recent clinical criteria, as will be outlined below [12, 13]. Among soft tissue sarcomas, anaplastic rhabdomyosarcoma has recently been shown to be particularly enriched in carriers of a germline *TP53* mutation [14]. Finally, among women with breast cancer and *TP53* mutations, 63–83% of the tumors have been reported to be HER2-positive [15, 16].

1.2 LFS: Biology and Pathogenesis

Two decades following its original clinical description, the underlying genetic alteration resulting in the Li-Fraumeni phenotype was discovered to be germline mutations in the *TP53* tumor suppressor gene [17]. Subsequent studies have documented the presence of a *TP53* germline mutation in approximately 70% of patients fulfilling the classic LFS criteria, up to 40% of patients meeting LFL criteria, and 29–35% of patients meeting the Chompret criteria [12, 18–20]. Up to 20% of *TP53* mutations occur de novo with the rest being inherited in an autosomal dominant fashion [21]. The prevalence in the general population of germline pathogenic *TP53* variants has recently been estimated, to be in the magnitude of 1 among 4500 individuals [22, 23, 24]. This finding highlights the need for further studies to investigate the cancer risk associated with rare *TP53* variants and the possibility of penetrance modifiers.

The lack of detectable germline *TP53* mutations in a proportion of families with LFS has led to efforts to identify other candidate genes. CHEK2 is a cell cycle checkpoint kinase which activates p53 in response to DNA damage. Germline mutations in *CHEK2* were originally described in a small number of LFS families; however, some of these mutations were subsequently shown to be polymorphisms, and many studies have since failed to demonstrate *CHEK2* as a major susceptibility gene for LFS [25–28]. The specific *CHEK2* c.1100delC mutation has been associated with hereditary breast cancer, but has not been found to play a major role in LFS, even in a large Dutch cohort with a high prevalence of this allelic variant [27, 29, 30]. Other candidate genes, including *PTEN*, *CDKN2*, *BCL 10*, *TP63*, and *BAX*, have also been shown not to have a causal role [31–35]. Therefore, to date, *TP53* is

the only gene involved in LFS. Methylation of the *TP53* gene promoter has been explored as a mechanism of epigenetic silencing, as occurs in hereditary non-polyposis colon cancer, but this was not found to be a frequent cause of LFS in families with no detectable *TP53* germline mutation [36].

The vast majority of *TP53* variants cluster in regions II to V, within the DNA binding domain, encoded by exons 5–8 (Fig. 1.2) [18, 37]. While most (>70%) are missense mutations, splice site, nonsense, frameshift, and intronic mutations have also been described, in addition to large partial or complete deletions of the gene or its promoter (Fig. 1.3) [20, 37, 38].

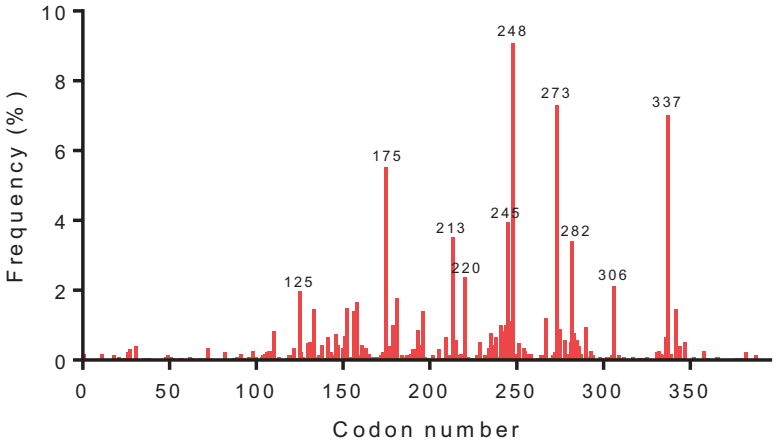

Fig. 1.2 Codon distribution and frequency of germline *TP53* mutations ($n = 2358$). Source [37]

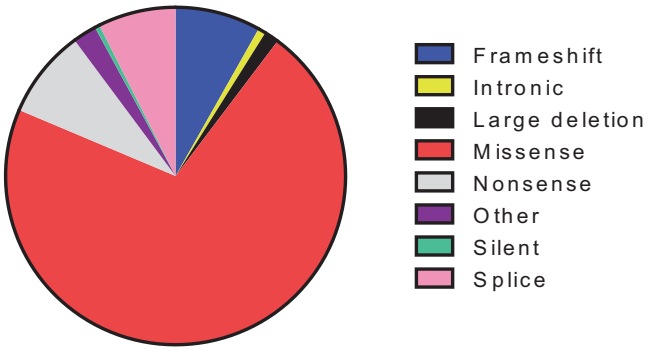

Fig. 1.3 Pie graph of germline *TP53* mutation types. Source [37]

1.3 Functional Studies and Genotype-Phenotype Correlations

Over 500 different germline variants have been identified with a mutational landscape spanning every exon and intron of the *TP53* gene [37]. Various studies have demonstrated that the type and location of the mutation differentially influence protein structure and function and thus the resulting malignant potential. Large-scale efforts to characterize the functional impact of *TP53* variants using saturation mutagenesis screens have uncovered diverse consequences ranging from complete loss-of-function to partial loss-of-function, dominant-negative activity over the wild-type protein to gain oncogenic potential, although the common biological impact of pathogenic *TP53* resulting in LFS is the alteration of the p53 transcriptional activity [39–41]. This functional diversity has been described as a "rainbow of mutants" (reviewed in [42]). Using a CRISPR-Cas9-based approach to generate a library of 8258 mutant alleles, it was found that cells harboring mutants with loss-of-function and dominant-negative activity have a fitness advantage over those that retain wild-type p53 function, suggesting a selective process that shapes the *TP53* mutational landscape and an explanation for the enrichment of hotspot mutations [40]. In a separate screen including nearly 10,000 *TP53* mutations, mutants with greater loss of function were associated with earlier age of first tumor onset in a retrospective analysis of germline carriers [39]. Functional analyses of EBV-immortalized lymphocytes and peripheral blood from LFS patients have shown that germline *TP53* pathogenic variants alter the p53-mediated transcriptional response to DNA damage. This constitutes a biological endophenotype of the syndrome (Zerdoumi et al. 2017), and a gradient can be observed; dominant-negative missense variants have a more drastic impact than the null variants or nondominant-negative missense variants such as the p.Arg337His Brazilian variant with incomplete penetrance.

Genotype-phenotype studies suggest that missense mutations in the core DNA binding domain are associated with earlier age of cancer diagnosis and higher cancer incidence, particularly for breast and brain tumors, compared with mutations resulting in protein truncation or inactivation [43]. Dominant-negative missense mutations were shown to be associated with an earlier mean age of first tumor onset (21.3 years) compared to other types of loss-of-function mutations (28.5 years) or *TP53* genomic rearrangements (35.8 years) [2]. These data support a "gain-of-function" potential for such missense mutations, including activation of cell cycle target genes by the mutant p53 protein, or alternatively a "dominant-negative" effect, whereby the mutant protein interferes with wild-type protein DNA binding (p53 acting as a tetramer)—both of which have previously been suggested in experimental studies, [39, 40, 44–47] as indicated above. Notably, retention of the wild-type *TP53* allele has been shown in two-thirds of tumors from patients carrying a missense mutation in the central DNA binding domain, demonstrating a reduction of selective pressure for loss of the wild-type allele [48]. This is in contrast to the invariable loss of heterozygosity associated with tumors from families harboring functionally null *TP53* germline mutations, which is more in keeping with "typical" tumor suppressor genetics [49].

Other studies have described tissue-specific genotype-phenotype correlations. Missense mutations in the L2 and L3 loops have been linked with CNS tumors, while mutations outside the DNA binding surface have been linked with adrenocortical carcinoma [10]. Of particular note, the unique Brazilian germline mutation at codon R337H, a low-penetrance (~10%) allele, has a striking association with adrenocortical carcinoma—conferring a remarkable 20,000-fold increased risk—although other LFS component tumors have occasionally been described in these families [50–52]. Several biological studies have shown that this variant has a less drastic impact on the transcriptional activity of p53 than the missense variants with dominant-negative activity. This explains the incomplete penetrance of this variant and its high incidence estimated to be 1/300 in south-west Brazil. In an analysis of pediatric adrenocortical carcinoma, individuals carrying mutations with higher functionality were less likely to have a strong family history of cancer, while those with lower functionality mutants had multiple primary malignancies and/or a positive family history [53]. The retention of transcriptional activity has also been associated with delayed tumor onset and prolonged lifetime cancer survival, particularly in males with glioma and gastric cancer [54]. The oligomerization of p53 is also a crucial factor in its function as a transcription factor and in cell fate decisions [55, 56]. In patients with mutations in the p53 oligomerization domain, carriers of multimeric mutants had significantly more favorable survival compared with carriers of OD variants resulting in monomeric p53 (median survival age 51 versus 33 years, respectively) [57].

Collectively, genotype-phenotype studies have demonstrated a link between the functional consequences and clinical impact of germline *TP53* mutations in terms of penetrance and cancer survival. However, there is currently no standardized classification method to distinguish between different *TP53* mutations for genotype-adapted recommendations. Further validation and continued collaboration will be necessary to establish a consensus for such risk-adapted guidelines that have the potential to improve clinical management in LFS.

1.4 Genetic Modifiers and Anticipation

The diversity of tumor types and presentations, and in particular in the age of tumor onset, within a given LFS kindred suggests the possibility of genetic and epigenetic modifiers as contributors to phenotypic variability. MDM2 is a negative regulator of p53 by targeting it for proteosomal degradation. The SNP309 T➔G variation increases MDM2 levels and thus potentially amplifies this effect. A number of studies have demonstrated accelerated tumor formation in *TP53* mutation carriers harboring the *MDM2* SNP309 polymorphism [58]. The mean age of tumor onset was found to be 10 years earlier in carriers of a G allele. This effect was amplified by the presence of the *TP53* codon 72 arginine (R) polymorphism, a variant which has a higher affinity toward MDM2, compared to the proline (P) variant [20, 59]. Similar findings were reported in a larger, more recent study of *TP53* mutation carriers with

MDM2 SNP 309, with a particularly pronounced effect in females, although the *TP53* codon 72P variant was associated with increased cancer risk [60]. Among *TP53* mutation carriers with *MDM2* SNP 309, accelerated tumor formation was more specifically and in some cases more dramatically documented in patients with soft tissue sarcoma and breast cancer, although these findings are limited by small numbers [61, 62]. More recently, an interaction between *MDM2* SNP 285 and 309 was documented in a series of 195 LFS patients: the 285G–309G haplotype was shown to be associated with a 5-year earlier age of tumor onset [63]. Furthermore, a p53-inducible microRNA (miR-605) that represses *MDM2* in a positive feedback loop was found to be a potential modifier of the LFS phenotype; the variant G-allele of miR-605 was associated with a 10-year earlier age of tumor onset [64].

The *TP53* PIN3 polymorphism, represented by a 16 bp duplication in intron 3, has also been shown to be a strong modifier of the germline *TP53* mutation phenotype. Its presence was associated with a 19-year difference in the mean age of tumor diagnosis among 25 *TP53* mutation carriers in a Brazilian study [61]. All patients who developed cancer before the age of 35 were found to be homozygous for the non-duplicated allele, and the modifier effect was particularly marked for soft tissue sarcoma (32.3-year difference). This finding was not restricted to those with the R337H *TP53* mutation. In contrast, a study of 152 germline mutation carriers reported that the duplication allele confers an increased cancer risk in men, and no effect was found on age of the first cancer diagnosis [65].

A genome-wide study of germline DNA copy number variation (CNV) in LFS families showed a significant increase in CNVs in germline *TP53* mutation carriers [66]. Furthermore, offspring were more likely to have increased CNVs compared to their parents, and those mutation carriers affected by cancer showed a trend for a greater number of CNVs compared to those carriers not affected by cancer. Together, these data may suggest an association between CNV frequency and severity of phenotype. The authors also demonstrated copy number variability in cancer-related genes in the LFS cohort and suggest that CNV changes are among the earliest manifestations conferred by *TP53* mutations and predispose to other genetic events leading to tumorigenesis. In a separate study, no evidence of CNV was observed in two successive generations of *TP53* mutation carriers and in successive generations of *Trp53*-deficient mice using whole-exome sequencing. Instead, the authors proposed a "genetic regression" model to explain anticipation in LFS, caused by the occurrence of rare SNP and de novo mutations rather than the accrual of CNVs [5].

Telomere attrition has also been studied as a mechanism leading to genetic anticipation, via increased genomic instability. Indeed, in families with LFS, telomere length was found to be shorter in individuals with cancer than in unaffected carriers, and the latter group were shown to have a faster rate of telomere attrition than normal controls [67, 68]. The occurrence of accelerated telomere attrition is one potential explanation for the earlier age of tumor onset observed in successive generations of a given family with identical phenotypes.

TP53 mutations have been observed in some tumors exhibiting a recently described mechanism of tumorigenesis termed *chromothripsis*, in which it is postulated that a single catastrophic event results in massive chromosome rearrangements

[69]. Whole-genome sequencing-based analysis of sonic hedgehog medulloblastoma (SHH-MB) from a patient with LFS revealed highly complex chromosome rearrangements. These findings were demonstrated in three additional SHH-MB patient samples and were associated with an amplification of known medulloblastoma oncogenes as a result of chromothripsis. Furthermore, 36% of other LFS-associated tumors tested from 11 LFS patients were shown to have rearrangements consistent with chromothripsis. The authors suggest that early *TP53* mutations may confer a state in cells that is permissive for chromothripsis and/or facilitates cell survival following such catastrophic DNA rearrangements. Interestingly, critical telomere shortening may be one mechanism by which chromothripsis occurs.

Together, identifying a composite of genetic changes in a series of biomarkers in carriers of *TP53* germline mutations can inform risk estimates for tumorigenesis and thus could be applied clinically in the context of surveillance practices recommended for these patients. However, current data require further validation, and the impact of the modifiers, characterized so far, is too weak to be applied in clinical settings.

1.5 Making a Diagnosis

A number of clinical definitions for LFS have been proposed, which form the basis for offering genetic testing to individuals (Fig. 1.4). The "classic" definition, based on a prospective analysis of 24 kindred, has been subsequently extended to more inclusive criteria ("Li-Fraumeni-like syndrome") described by Birch and Eeles and then the Chompret criteria which include a subset of patients to be tested even in the absence of a suggestive family history [12, 70–74]. These criteria for *TP53 t*esting have been sequentially updated and correspond to different clinical situations: (a) a proband with one LFS tumor (soft tissue sarcomas, osteosarcomas, adrenocortical carcinomas (ACC), central nervous system (CNS) tumors, and very early-onset female breast cancers) before the age of 46 years and at least one first- or second-degree relative with one LFS tumor before the age of 56 years; OR (b) a patient with two LFS primary tumors, the first being diagnosed before the age of 46 years, independently of the familial history; OR (c) a child with ACC, choroid plexus tumor, anaplastic rhabdomyosarcoma, hypodiploid acute lymphoblastic leukemia, or sonic hedgehog-driven medulloblastoma, independently of the familial history; OR (d) a female with breast cancer diagnosed before the age of 31 years, independent of the familial history. Finally, bronchoalveolar lung cancer is added to the core tumor types. Regardless of familial history, the mutation detection rate of disease-causing germline *TP53 v*ariants has been estimated to be 50–80% in children presenting with ACC (or choroid plexus carcinomas) [75]; up to 73% in children with rhabdomyosarcoma of embryonal anaplastic subtype and between 3% and 8% in females with breast carcinoma before 31 years of age (reviewed in [76] and [77]) [12, 77–79]. The guidelines for Genetic/Familial High-Risk Assessment, the National Comprehensive Cancer Network (NCCN) suggests that women with early-onset

Criteria	Description
Classic[70]	A proband with sarcoma diagnosed under age 45 years, ***and*** A first-degree relative with any cancer under 45 years, ***and*** Another first- or second-degree relative with either any cancer under 45 years, or a sarcoma at any age
Birch[71]	Among families that do not conform to classic LFS: A proband with any childhood cancer or sarcoma, brain tumor, or adrenocortical carcinoma diagnosed under 45 years, ***and*** A first- or second-degree relative with a typical LFS-related cancer (sarcoma, breast cancer, brain tumor, leukemia, or adrenocortical carcinoma) diagnosed at any age, ***and*** A first-or second-degree relative in the same genetic lineage with any cancer diagnosed under 60 years
Eeles[72]	Among families that do not conform to classic LFS: Two different tumors that are part of extended LFS in first- or second-degree relative at any age (sarcoma, breast cancer, brain tumor, leukemia, adrenocortical tumor, melanoma, prostate cancer, and pancreatic cancer)
Chompret[73,74]	Proband with sarcoma, brain tumor, breast cancer, or adrenocortical carcinoma before age 36 years, ***and*** At least one first-or second-degree relative with cancer (other than breast cancer if the proband has breast cancer) under 46 years ***or*** A relative with multiple primaries at any age ***Or*** A proband with multiple primary tumors, two of which are sarcoma, brain tumor, breast cancer, and/or adrenocortical carcinoma, with the initial cancer occurring before the age of 36 years, regardless of family history ***Or*** A proband with adrenocortical carcinoma at any age, regardless of family history
Revised Chompret[13]	A proband with tumor belonging to LFS tumor spectrum (soft tissue sarcoma, osteosarcoma, brain tumor, premenopausal breast cancer, adrenocortical carcinoma, leukemia, lung bronchoalveolar cancer) before 46 years, ***and*** At least one first- or second-degree relative with LFS tumor (except breast cancer if proband has breast cancer) before age 56 years, ***or*** with multiple tumors ***Or*** Proband with multiple tumors (except multiple breast tumors), two of which belong to LFS tumor spectrum and first of which occurred before age 46 years ***Or*** Patient with adrenocortical carcinoma or choroid plexus tumor, irrespective of family history

Fig. 1.4 Clinical criteria for LFS

breast cancer (≤35 years) are a group who may be considered for testing, regardless of family history [76]. Therefore, familial history of cancer is not mandatory when considering genetic testing of *TP53*, and this corresponds to one of the most important evolutions in the field.

1.6 Implications of a Diagnosis of LFS

1.6.1 Surveillance

The remarkably high lifetime risk of cancer in patients with LFS implies an imperative for close surveillance of affected family members to facilitate early detection and management of neoplasms. Until recently, this practice has been discouraged owing to lack of data supporting its effectiveness; in addition, the historical unpredictability of age and site of tumor onset makes clinical surveillance strategies more complex than in other cancer predisposition syndromes. The feasibility of a clinical surveillance protocol for patients with LFS has been described in an 11-year prospective observational study (Fig. 1.5), which demonstrated a survival advantage using this approach [80, 81]. These recommendations are being adopted by numerous centers around the world, and components of this protocol have been incorporated as recommended practice guidelines set forth by the National Comprehensive Cancer Network [76]. Several international studies, mostly performed without gadolinium-based contrast agents (GBCAs), have confirmed the efficiency of WBMRI in terms of tumor detection, with an overall estimated detection rate of 7%

Figure 5: Surveillance strategy for individuals with germline *TP53* mutations

Tumor Type	Surveillance Strategy
Children	
Adrenocortical carcinoma	▪ Ultrasound of abdomen and pelvis every 3-4 mths ▪ 24h urine cortisol, if feasible ▪ Bloodwork every 3-4 months:* 17-OH-progesterone, total testosterone, dehydroepiandrosterone sulfate, androstenedione
Brain tumor	▪ Annual MRI of the brain
Soft tissue and bone sarcoma	▪ Annual total body MRI
Leukemia/lymphoma	▪ Bloodwork every 3-4 mths: complete blood count , erythrocyte sedimentation rate, lactate dehydrogenase
General assessment	▪ Complete physical examination every 3–4 mths, including anthropometric measurements plotted on a growth curve (with particular attention to rapid acceleration in weight or height), signs of virilisation (pubic hair, axillary moisture, adult body odour, androgenic hair loss, clitoromegaly, or penile growth), and full neurological assessment ▪ Prompt assessment with primary care physician for any medical concerns
Adults	
Adrenocortical carcinoma (age 18-40y)	▪ Ultrasound of abdomen and pelvis every 3-4 mths ▪ Blood tests every 3–4 months:* 17-OH-progesterone, total testosterone, dehydroepiandrosterone sulfate, and androstenedione ▪ 24h urine cortisone, if feasible
Breast cancer	▪ Monthly Breast Self Examination starting at age 18y onwards ▪ Clinical breast exam twice a year starting at age 20-25y, or 5-10y before the earliest known breast cancer in the family ▪ and breast MRI screening starting at age 20-75y, or individualized based on earliest known breast cancer in family ▪ Consider risk-reducing bilateral mastectomy
Brain tumor (age 18y onwards)	▪ Annual MRI of the brain

Fig. 1.5 Surveillance strategy for individuals with germline *TP53* mutations

Soft tissue and bone sarcoma (age 18y onwards)	▪ Annual total body MRI ▪ Ultrasound of abdomen and pelvis every 3-4 mths
Colon cancer	▪ Biennial colonoscopies beginning at age 25y, or 10y before the earliest known colon cancer in the family
Melanoma (age 18y onwards)	▪ Annual dermatology examination
Leukemia/Lymphoma (age 18y onwards)	▪ Blood tests every 3-4 mths: complete blood count, erythrocyte sedimentation rate, lactate dehydrogenase
General assessment	▪ Complete physical exam every 3-4 mths ▪ Prompt assessment with primary care physician for any medical conditions

*Serial specimens obtained at the same time of day and processed at the same laboratory. †Breast ultrasound with mammography as indicated by breast density, but not instead of breast MRI with mammography. ‡Breast MRI to alternate with total body MRI (one scan every 6 mths).

Source:[81]

Fig. 1.5 (continued)

for new and localized primary cancers on a first prevalent screen. Of note, surveillance practices should be continued beyond the detection of a first cancer, as patients with LFS are at high risk for multiple primary neoplasms over their lifetime [7, 70, 74]. Another surveillance strategy using PET-CT has also been described; however, concerns regarding radiation exposure may hamper its widespread use in this population [82].

The development of a surveillance protocol represents a significant advance in the care of patients with LFS [83, 84]. Indeed, as mentioned above, patient-specific modifications to the surveillance approach will be possible once the influence of other genetic and epigenetic biomarkers becomes clearer. More work is needed in this area to assess long-term outcomes, psychosocial, compliance, and economic impact. One recent report describes almost 80% adherence to varied surveillance recommendations given to high-risk LFS individuals, with the large majority of patients reporting feeling a sense of control and security as a result of their participation [85]. An important challenge will be the international dissemination and implementation of this surveillance protocol which will depend, in each country, on health system organization. Some of these studies also incorporate an assessment of psychosocial impact, which will be an important additional measure to consider when defining management strategies for this patient population.

1.6.2 Genetic Testing

The availability of surveillance guidelines which show early but promising suggestions of efficacy lends support for genetic testing of at-risk individuals among family members once a diagnosis is made in an index case. An argument can be made to extend this practice to pediatric patients as well, since they face a considerable cancer risk—an estimated 15–40% risk in the first two decades of life, depending in

part on the ascertainment method of the study [8, 86]. The high potential for disease onset within the pediatric age group and the recent suggestions of benefit from early intervention (i.e., surveillance) together meet the requirements of most regulatory bodies on the ethical appropriateness of pre-symptomatic genetic testing of minors [76, 87–94].

The benefits and risks of predictive genetic testing require careful consideration [88, 90, 95, 96]. The confirmation of a negative test can cause an individual significant emotional relief regarding risk to self and offspring and the avoidance of unnecessary testing and interventions. Identification of a mutation (positive test result) can stimulate preparation and adjustment in life planning regarding educational and occupational goals, social circumstances, support systems, and reproductive planning. The resolution of uncertainty, irrespective of test results, is described as a positive outcome for many patients as well. Importantly, genetic testing allows for the option of earlier surveillance and preventative strategies. The possibility of negative psychosocial consequences is also highlighted by a number of studies [97–99]. These include heightened feelings of anxiety and preoccupation with the idea of cancer when new symptoms arise, the onset of guilt, depression, and denial in some. There may be changes in family dynamics; feelings of "survivor guilt" in those with negative results, the emergence of the "vulnerable child syndrome" in young family members with positive results, and loss of self-esteem in pediatric patients themselves. Inappropriate stigmatization and discrimination may have harmful consequences for educational, employment, and social integration.

A review of studies specifically assessing the impact of genetic testing of individuals at risk for more common inherited cancer syndromes, including breast cancer susceptibility and hereditary non-polyposis colon cancer (HNPCC), reported that non-carriers derived significant psychosocial benefits. Among carriers, no adverse effects were found in most studies, but a few did document short-term cancer-related anxiety [100]. Response to genetic testing among those affected by cancer is mediated by an individual's personal experiences with cancer. Psychosocial consequences of genetic testing specifically in the context of LFS has also been reviewed [101]. In general, among 18 families with germline *TP53* mutations who completed self-report questionnaires, an unfavorable genetic test result was not found to cause adverse psychological effects. Approximately one quarter of participants reported clinically relevant levels of distress, irrespective of their choice to undergo genetic testing or their personal cancer history. Higher levels of LFS-related distress was reported by women, those with high perceived risk of developing cancer, and those with perceived lack of social support. A follow-up study documented a similar prevalence of distress in partners of high-risk individuals; furthermore, symptoms between partners appear to correlate [102].

The above factors influence the uptake of genetic testing among at-risk individuals. Previous studies have documented a 25–40% uptake of genetic testing among individuals at risk for LFS (a decision aid was employed in a study documenting 40% uptake). [103–105] In a more recent study [101], 55% of 119 individuals at risk agreed to pre-symptomatic genetic testing, which is comparable to studies conducted in other hereditary cancer syndromes [106, 107]. Most parents opt in favor

of *TP53* testing for their own children based on findings from a study including 49 parents interviewed across 7 different sites [108]. The most common motivations for genetic testing were to obtain certainty of cancer risk and to estimate risk for children, although some patients reported a desire to plan regular surveillance [101]. The interval proposal of a promising surveillance protocol will likely improve uptake of predictive genetic testing among patients at risk for LFS.

The controversial issues of pre-implantation genetic diagnosis (PGD), prenatal diagnosis (PND), and newborn screening for inherited cancer predisposition syndromes have been discussed in recent years [109–112]. A French survey of cancer geneticists and multidisciplinary prenatal diagnosis teams reported >80% acceptability of PGD/PND in the circumstance of severe childhood-onset cancers which have no effective method of prevention or treatment [112]. LFS was considered to be part of this category and was the one inherited cancer syndrome for which cancer geneticists rated PGD/PND to be most acceptable. It should be noted that this study was completed before the recent work on clinical surveillance was published. A Dutch study evaluating family members with a hereditary cancer syndrome, including 18 LFS families, found 38% had a positive attitude toward PGD, as assessed by a self-report questionnaire [110]. No association was found between attitudes and one's personal/family history with LFS or with cancer-related distress, risk perception, or feelings of guilt. While the ethical and psychosocial burden of these practices can be quite significant, some authors note that the prospect of PGD can improve motivation for genetic testing among individuals at risk and, furthermore, can offer families a proactive means of minimizing the cancer risk they pass on to their children, thereby alleviating some of their significant burden [109].

1.6.3 Treatment Implications

Current treatment approaches to malignancies in the context of LFS are not different from standards of care; however, specific vulnerabilities of this patient population deserve consideration. It is generally accepted that radiation therapy should be used judiciously or avoided if possible for patients with LFS, as the risk of radiation-induced second malignancies is notable [7, 9, 18, 70, 74, 113]. There are growing arguments supporting the notion that genotoxic treatments, and in particular radiotherapy, contribute to the development of second primary tumors, which is remarkably high – up to 50% in *TP53* variant carriers. Indeed, a number of studies of LFS patients with early-onset breast cancer demonstrate second malignancies in the radiation field, in particular sarcoma, prompting some authors to suggest careful consideration of the risk/benefit ratio of radiation use, and potential bilateral mastectomy in place of adjuvant radiotherapy in this population [114, 115]. Preclinical data also support this finding, and studies of fibroblasts or lymphoblastoid cells from patients with LFS demonstrate reduced p53-mediated transcriptional response to genotoxic agents, enhanced structural chromosomal changes in response to irradiation, and in some lines increased longevity/decreased apoptosis and

radioresistance [116–119]. The caution is further substantiated by the well-recognized strikingly reduced time to tumor formation in *Tp53*$^{+/-}$ and *Tp53*$^{+/m}$ mice exposed to whole body radiation. Enhanced survival of genetically damaged cells is the proposed end result of a failure of p53 to induce cell cycle arrest and senescence in response to DNA strand breaks. Of note, chemoresistance has also been demonstrated in preclinical and clinical studies of sporadic LFS-associated cancers harboring *TP53* mutations (as reviewed in [120]), which may suggest a similar finding for patients with germline mutations.

Targeted therapeutic strategies have also recently been explored in the context of LFS [42]. The pervasive involvement of p53 in the pathogenesis of sporadic tumors has catalyzed interest in the development of agents to modulate p53 in cancer cells and reactivate its normal function [120]. Preliminary data is available for Advexin® (Introgen Therapeutics Inc., TX, USA), an adenovirus containing a p53 expression cassette, which facilitates transfer of a wild-type *TP53* gene into malignant tissue. A number of clinical trials have been conducted using this approach in patients with sporadic tumors that harbor *TP53* mutations, as monotherapy or in combination with conventional chemotherapy, with some evidence of clinical responses [120, 121]. There is a single-case report of its use in a patient with LFS and progressive, refractory embryonal carcinoma. Other delivery systems including liposomal encapsulated wild-type *TP53* are being explored and show tumor growth inhibition in preclinical solid tumor models [121]. A number of other strategies, including restoration of wild-type function to mutant p53, stabilization of p53, selective degradation of mutant p53, and activation of other p53 family members, are being investigated [120].

A growing body of preclinical research linking p53 to cellular metabolism and the use of metabolic regulatory drugs (such as rapamycin and metformin) have shown early promise as cancer prevention strategies [122, 123]. Recent findings of increased oxidative metabolism in LFS patients and a report that metformin could inhibit mitochondrial respiration and delay tumor onset in *Trp53*-deficient mice have sparked interest in its use, and a clinical trial is in development [124, 125]. Finally, the notion of chemoprevention is one which may be applied in the future to patients with LFS. Recent studies have reported a high frequency of HER2-positive breast cancer and germline *TP53* mutations [15, 16]. HER2 amplification/overexpression was documented in 67–83% of women with germline mutations, compared to 16–25% of control patients with early-onset breast cancer, while the frequency of ER- and PR-positive tumors was not statistically different. This association raises the possibility of a chemopreventive strategy using HER2-targeted agents in women with LFS.

1.7 Conclusion

Li-Fraumeni syndrome is a genetically and phenotypically heterogeneous cancer susceptibility disorder that has emerged as one of the most compelling paradigms for the need for expanded collaborative multi-institutional efforts to better

understand its pathophysiology, opportunities for early tumor detection, and clinical management. With the advent of next-generation sequencing technologies, it is likely that rapid multi-gene platform technologies will emerge that will facilitate more accurate prediction of age-of-onset and tumor type in *TP53* mutation carriers. Together with more sensitive and rapid functional assays, these combined technologies will revolutionize and personalize the clinical management of these patients. Improvements in the specificity of surveillance strategies and innovative approaches to chemoprevention or therapy that takes target aberrations of p53 function represent the greatest challenges on the immediate horizon for these patients. Coupled with improved understanding of the psychosocial impact on families afflicted with LFS, it is anticipated that more effective and meaningful management of patients will be available in the not-too-distant future.

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Chapter 2
Pediatric Central Nervous System Cancer Predisposition

Anirban Das and Uri Tabori

Abstract Malignancies of the central nervous system are the second most common cause of cancers in children and adolescents. The explosion of molecular diagnostics in pediatric neuro-oncology has contributed to the growing recognition that several of these cancers may be associated with a germline predisposition. These include well-characterized entities like Li-Fraumeni and neurofibromatosis syndromes, as well as conditions where knowledge is still evolving, like the germline DNA replication repair deficiency syndromes. Some syndromes are associated with specific cancer types, while in others several types of brain cancers can develop, either synchronously or over time. Certain syndromes include specific cancer entities as the predominant manifestation. In such cases, cancer genetics can guide evaluation for germline predisposition. Other conditions may include more prominent systemic manifestations to indicate an underlying inherited condition. Timely diagnosis directly impacts management in most cases, allows screening of family members, and benefits the patient and the kindred by institution of surveillance programs. In this chapter, we summarize the recent advances in diagnosis and management for these increasingly recognized conditions, several of which are further expanded upon in other chapters in this text.

Keywords Central nervous system · Brain tumors · Medulloblastoma · Glioma · Gorlin syndrome · Constitutional mismatch repair deficiency · Choroid plexus tumor

A. Das · U. Tabori (✉)
Division of Hematology/Oncology, The Hospital for Sick Children, Toronto, ON, Canada
e-mail: Uri.tabori@sickkids.ca

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2.1 Introduction

Brain tumors are the second most common cause of cancer in children. These tumors are the leading cause of death and long-term morbidity due to their poor outcome and the use of aggressive therapeutic modalities to achieve cure. Early detection and awareness can therefore have an impact on the management and long-term outcome of these children.

Leukemias, which are the most prevalent pediatric cancers, are infrequently associated with genetic syndromes (see Chap. 11). Brain tumors, on the other hand, are commonly associated with predisposing germline mutations and in some specific cases more than half of the children affected by a certain type of brain tumor will harbor a germline mutation in that particular corresponding gene. Brain tumors are therefore by far the most common neoplasms associated with cancer predisposition in childhood, and management of these patients and families is frequently affected by these underlying conditions.

One can group these predisposing syndromes by tumor type, which will inform the physician as to which syndromes to consider when a child is affected by a specific tumor. Another option is to divide these conditions into two groups: syndromes where cancer is the only manifestation, and syndromes in which additional phenotypes exist that can assist in early detection of carriers.

For this chapter, we chose to divide the syndromes into those that are associated with several tumor types, and those in which a germline mutation is generally associated with a specific type of brain tumor as the major clinical manifestation. We will discuss the conditions which are more commonly seen, and more rare manifestations will be mentioned in the Table 2.1.

2.2 Syndromes Associated with Multiple Cancers

In these syndromes, the mutated gene is associated with cancers in multiple organs and at different ages. Surveillance and counseling are more extensive than just brain tumor management.

2.2.1 Li-Fraumeni Syndrome

Li-Fraumeni syndrome (LFS) is an autosomal dominantly inherited cancer predisposition syndrome affecting 1 in 5000 to 10,000 individuals (see Chap. 1). It is the prototypical syndrome causing cancer in multiple sites at different ages. Individuals with the disease have a lifetime risk of 85–100% of developing cancer, with a 20–30% risk before the age of 30. Originally described by Li and Fraumeni in 1969 [1] as a familial breast, soft tissue sarcoma, and brain tumor predisposition

Table 2.1 Inheritable syndromes predisposing to central nervous system neoplasms

Syndrome	Genetics	Non-neoplastic characteristics	Cancers	Comments
Brain tumor-polyposis syndrome type 2 (FAP syndrome)	Autosomal dominant	Colorectal polyposis (numerous, small, late onset). Gastric fundic polyps. Congenital hypertrophy of the retinal pigment, epidermal inclusion cysts, dental anomalies, and osteosclerotic jaw lesions (Gardner syndrome-like).	Medulloblastoma. Colon cancer, hepatoblastoma, aggressive fibromatosis.	Surveillance protocol available.
Fanconi anemia and homologous repair deficiency syndromes	Multiple genes in the pathway. Mainly, *BRCA2* and *PALB2*. Autosomal recessive. 3:/One million individuals.	*Congenital malformations:* Abnormal thumbs, short stature, microcephaly, abnormal skin pigmentation: café au lait spots. *Bone marrow failure:* Anemia, thrombocytopenia, leucopenia.	Medulloblastoma. Multiple carcinomas in family members. Wilms tumor, breast cancer, pancreatic cancer.	
Gorlin syndrome (basal cell nevus syndrome; nevoid basal cell carcinoma syndrome)	*PTCH1*, *Sufu*. Autosomal dominant. 1:50,000–150,000 individuals	*Superficial malformations*: Jaw cysts, palmar/plantar pits. *Radiologic manifestations*: Calcification of the falx cerebri and tentorium cerebelli, bridging of the Sella turcica. Bone anomalies: Spine abnormalities, rib abnormalities, metacarpal/phalangeal abnormalities.	Medulloblastoma. Meningioma. Basal cell carcinoma of skin.	Surveillance protocol available. **Targeted therapies with SHH inhibitors exist.**
Li-Fraumeni syndrome	*TP53* gene. Autosomal dominant. 1:5,000–20,000 individuals.	None	Gliomas, choroid plexus carcinomas, medulloblastoma. Multiple tumors in family members: Breast cancer, brain tumors, sarcomas, adrenocortical carcinoma, and others.	Surveillance protocol available.

(continued)

Table 2.1 (continued)

Syndrome	Genetics	Non-neoplastic characteristics	Cancers	Comments
Multiple hamartoma syndrome (Cowden syndrome)	*PTEN*. Autosomal dominant. 1:200,000 individuals.	*Mucocutaneous manifestations:* Trichilemmomas, acral keratoses, oral mucosal papillomatosis, palmoplantar keratoses. Hamartomas of gastro-intestinal tract, bones, brain, eyes, and genitor-urinary tract.	Dysplastic ganglioglioma of the cerebellum. Family members with breast carcinoma thyroid follicular carcinoma, endometrial carcinoma, renal cell carcinoma.	
Neurofibromatosis type 1	*Neurofibromin* gene (17q11.2). Autosomal dominant. 1:3000 individuals.	*Cutaneous manifestations*: Café au lait spots or hyperpigmented macules (six or more, ≥ 5 mm diameter if <10yo or ≥ 15 mm in adults; axillary or inguinal freckling; subcutaneous or cutaneous neurofibromas; subcutaneous plexiform neurofibromas +/− overlying hyperpigmentation or hypertrichosis. *CNS manifestations:* Learning difficulties +/− ADHD; mild/moderate developmental delay; cerebrovascular lesions-ectasias, stenoses, aneurysms, Moyamoya disease; macrocephaly (common); precocious puberty-idiopathic. *Ocular manifestations:* Multiple iris hamartomas (Lisch nodules). *Osseous manifestations:* Long bone intramedullary fibrosis, bowing or tibial pseudo-arthrosis; cortical thinning; vertebral dural ectasia; sphenoid bone dysplasia; scoliosis +/− kyphosis. *Other manifestations:* Reno-vascular hypertension; coronary artery aneurysms Constitutional short stature (common).	Low- and high-grade gliomas, plexiform and other neurofibromas, malignant peripheral nerve sheath tumor, chronic myelomonocytic leukemia, rare: Sarcomas, neuroblastoma, pheochromocytoma.	Surveillance protocol available.

Syndrome	Genetics	Non-neoplastic characteristics	Cancers	Comments
Neurofibromatosis type 2	*NF2 (Merlin)* gene (22q12.2). Autosomal dominant. 1:37,000 individuals.	*Ocular manifestations:* Posterior sub-capsular "juvenile" cataracts; retinal hamartomas; epiretinal membranes.	Acoustic neurinoma, multiple CNS schwannomas and meningiomas, and low-grade spinal ependymomas.	Surveillance protocol available. Targeted therapy with bevacizumab
Mismatch repair cancer syndrome (brain tumor-polyposis syndrome type 1; "Turcot syndrome")	*MLH1 MSH2*, *MSH6*, *PMS2*. Autosomal recessive. Unknown.	Colorectal adenomas (few, large, early onset) without polyposis. Café au lait spots, Lisch nodules, axillary freckling (neurofibromatosis type 1-like characteristics). Hepatic focal nodular hyperplasia.	Gliomas, medulloblastoma, and embryonal tumors. Gastrointestinal cancer and lymphoma/leukemia.	Surveillance protocol available.
Rhabdoid predisposition syndrome	*SMARCB1* and *SMARCA4*. Autosomal dominant. Unknown: <1:200,000 Individuals.	None	CNS atypical teratoid Rhabdoid tumor Renal and extra-renal rhabdoid tumor. Rare and emerging: sarcomas, meningioma	Surveillance protocol available.

(continued)

Table 2.1 (continued)

Syndrome	Genetics	Non-neoplastic characteristics	Cancers	Comments
Tuberous sclerosis complex (Bourneville disease)	*TSC1*, *TSC2*. Autosomal dominant. 1:5800–30,000 individuals.	Hypomelanotic "ash-leaf" macules, facial angiofibromas ("adenoma sebaceum"), ungual fibromas, periungual fibromas (Koenen tumors), forehead plaque fibromas, truncal connective tissue nevus ("shagreen patch"), dental enamel pits, gingival fibromas. *Ocular manifestations:* Multiple retinal nodular hamartomas (phakomas), hypopigmented iris spots. *CNS manifestations:* Epilepsy, autism spectrum; cortical and sub-cortical tubers, subependymal nodules. *Other manifestations:* Bone cysts, pulmonary cystic lymphangiomatosis (recurrent pneumothoraces, dyspnea, cor pulmonale, respiratory failure).	Subependymal giant cell astrocytomas. Renal angiomyolipoma, cardiac rhabdomyoma, pulmonary lymphangioleiomyomatosis, and renal cell carcinoma.	Surveillance protocol available. Targeted therapy with mTOR inhibitors.
von Hippel-Lindau syndrome	*VHL* gene. Autosomal dominant. 1:36,000 individuals.	Hearing loss/tinnitus (endolymphatic sac cysts). Cardiovascular and cerebrovascular disease. Hypertension.	Hemangioblastomas of the brain and spine. Hemangioblastomas in other locations, retinal hemangioblastomas, renal cell carcinomas and cysts Pheochromocytoma, pancreatic tumors and cysts, epididymal and broad ligament cystadenomas, and endolymphatic sac tumors.	Surveillance protocol available.

syndrome, we know now that this is not an organ-specific disease and that individuals have a risk of developing cancer in many other organs including the development of rare tumors such as adrenocortical carcinomas, as well as hematologic malignancies.

Molecular genetics: In 1990, the association between LFS and germline mutations in the tumor suppressor *TP53* gene was made [2]. *TP53*, the "gatekeeper of the genome," is located on chromosome 17p13.1 and is one of the major proteins that control genome integrity after DNA damage, hypoxia, and other stressors. *TP53* activation results in cell cycle arrest, senescence, and apoptosis. We now know that *TP53* is involved in many other cancer-associated pathways in the cell. More than 50% of adult tumors possess *TP53* mutations, and greater than 80% of adult high-grade gliomas have disruption of the *TP53* pathway [3].

Three brain tumors are associated with LFS: gliomas, choroid plexus carcinoma, and medulloblastoma [4]. Choroid plexus tumors affect LFS carriers in the first decade of life and medulloblastoma usually in the second decade, while malignant gliomas occur throughout childhood but more commonly seen in young adults.

Choroid plexus carcinomas are one of the most common presentations of LFS in young children and a part of the criteria for the diagnosis of the syndrome [4]. Furthermore, a significant proportion of patients, perhaps more than 50% of children with choroid plexus carcinoma, will harbor germline *TP53* mutations. Somatic mutations in *TP53* are observed in up to 50% of choroid plexus carcinomas, and this confers a worse survival for these patients [5]. This phenomenon may be caused by resistance of *TP53* mutant tumors to radiation and chemotherapy [6].

Five to 10% of **medulloblastoma** harbor somatic *TP53* mutations. These are strictly confined to the WNT and SHH subgroup of tumors [7–11]. This observation has resolved several controversies regarding the survival disadvantage of these mutations for these patients [8, 12]. *TP53* mutations do not alter the excellent survival of patients with WNT-medulloblastoma, as these are downstream, and not driver or germline events. On the other hand, *TP53* mutant SHH medulloblastomas are commonly seen in the second decade of life and have unfavorable outcome [7, 10]. A significant proportion of childhood SHH medulloblastoma will harbor both somatic and germline *TP53* mutations [13]. SHH medulloblastoma from LFS patients exhibits a unique pattern of genetic/genomic alterations suggesting chromothripsis as an initiating event [14].

Gliomas have been recognized as part of the LFS spectrum from the earliest reports [1]. Although TP53 expression is associated with worse outcome in pediatric glioblastoma [15], currently no data exist regarding the significance of germline *TP53* mutations in pediatric high-grade gliomas. The surveillance protocol [16] developed by the group at The Hospital for Sick Children in Toronto, Canada, uncovered several low-grade gliomas suggesting that some of LFS-associated glioblastomas arise as secondary glioblastomas and may benefit from early intervention.

Clinical implications: Current recommendations are to screen any child with choroid plexus carcinoma, SHH medulloblastoma, and patients with high-grade gliomas with a family history of LFS tumors for germline *TP53* mutations [13]. Surveillance protocols have been developed for individuals with LFS revealing a

high rate of early tumor detection and consequently increased chances of curative intervention [17]. Recently, striking survival benefit for children has been observed using these protocols especially due to early detection of brain tumors. Although no targeted therapy for *TP53*-mutated tumors is currently available, detection of *TP53* mutations in the tumor and in the germline has significant prognostic and therapeutic implications. Although still a subject of debate, both children and adults with LFS have been considered exquisitely sensitive to ionizing irradiation, with a substantially increased likelihood of developing irradiation-induced secondary malignancies [18, 19]. Secondary myelodysplastic syndrome following specific chemotherapies has also been reported in LFS carriers [20].

2.2.2 *Neurofibromatosis Type I (NF1, von Recklinghausen's Disease)*

NF1 is the most common CNS cancer predisposition syndrome known to date (see Chap. 4). It is an autosomal dominant condition with a worldwide incidence of 1 per 2500–3000 individuals [21]. Importantly, this is a multi-system condition, and the diagnosis is generally based on clinical criteria [21–23]. Individuals with NF1 have significant morbidity and early mortality not necessarily due to cancer predisposition [24]. The nervous system is commonly affected in NF1 patients, and most cancers are of nervous system origin including gliomas, benign neurofibromas, and malignant nerve sheath tumors. However, other rare cancers including chronic myelomonocytic leukemia, certain endocrine tumors, rhabdomyosarcoma, and neuroblastoma are reported in patients with this condition [25].

Gliomas: The most common central nervous system tumors in NF1 are optic pathway gliomas (OPG) affecting up to 15% of children with the syndrome [21]. Conversely, up to a third of children with OPG have germline mutations in *NF1*. Bilateral optic nerve gliomas exist almost exclusively in children with NF1 (Fig. 2.1a).

NF1-related OPG have a generally indolent course, often with spontaneous growth arrest. Indeed, the vast majority of these OPG will not progress after initial diagnosis. Up to 15% of these tumors progress resulting in visual loss among other symptoms and thus require intervention. High-grade gliomas are relatively uncommon but have been reported and should be considered in patients whose tumors arise in an uncharacteristic location or demonstrate particularly aggressive behavior [26, 27].

Children with NF1 often exhibit multiple lesions mainly in the basal ganglia and brainstem which are difficult to assess. These include T2-enhancing lesions which are not causing edema or pushing other tissues and termed FLAIR-associated subcortical intensities (Fig. 2.1b). These lesions tend to grow and disappear and rarely cause symptoms. Differentiating between FLAIR-associated sub-cortical intensities

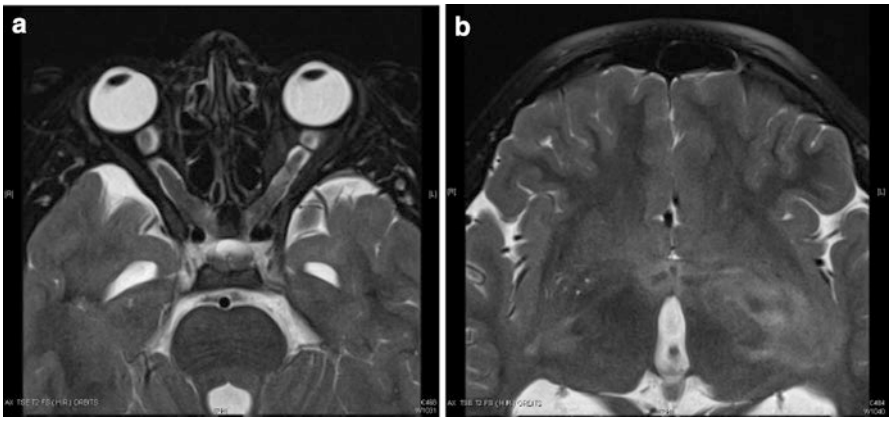

Fig. 2.1 Typical imaging findings of an NF1 patient. (**a**) Widened and convoluted bilateral optic nerves which are pathognomonic for NF1. (**b**) T2 coronal view reveals bilateral high-intensity sub-cortical lesions

and low-grade gliomas in NF1 may be challenging, and sometimes biopsies need to be taken to make the distinction.

Molecular genetics: *NF1* results in loss of function of the tumor suppressor protein neurofibromin. This large protein is a key negative regulator of the RAS pathway by catalyzing the hydrolysis of active guanosine triphosphate-bound RAS (RAS-GTP) to inactive guanosine diphosphate-bound RAS (RAS-GDP) [28]. Dysfunctional neurofibromin results in constitutive activation of downstream oncogenic pathways including *MAPK* and *mTOR*. Mutations or deletions in the *NF1* gene can be identified in more than 85% of individuals with NF1. However, since the gene is very large and difficult to analyze, diagnosis and management currently are still generally based on clinical criteria. Recently, a genotype-phenotype association with OPG and mutations in the 5′ third of the gene was reported [29]. If this holds true in subsequent studies, it may affect the surveillance protocol (see below).

Management: Since NF1 is a multi-system condition, careful monitoring is recommended in multidisciplinary clinics [21]. The markedly variable clinical manifestations as well as variability in tumor occurrence make recommendations for follow-up difficult to achieve. Optic gliomas affecting both optic nerves and coexistence of FLAIR-associated sub-cortical intensities should raise a suspicion of NF1, and genetic counseling is recommended. Unfortunately, surveillance neuroimaging in asymptomatic children has not been shown to reduce the incidence of visual loss in this population, and frequent neuro-ophthalmologic examination remains standard of care [30]. For FLAIR-associated sub-cortical intensities and other atypical brain lesions, close monitoring is recommended and treatment should be reserved for progressive disease. Individuals with NF1 are particularly sensitive to the damaging effects of ionizing irradiation, leading both to an increased incidence of irradiation-induced cancers [31] and to cerebrovascular damage (Moyamoya syndrome) [32, 33]. Thus, NF1 children with OPG in particular and brain tumors in

general should avoid cranial irradiation apart from highly exceptional circumstances. OPG in the context of NF1 have superior progression-free survival compared to their non-NF1 counterparts. Therefore, patients with NF1 and OPG should be treated conservatively at least initially and even in progressive/recurrent tumors [34, 35].

Perhaps the most significant advance in the management NF1 patients is the high efficacy of MEK inhibitors for the treatment of both plexiform neurofibromas [36] and low-grade gliomas [37]. MEK inhibitors reveal significant response rate not seen before with chemotherapy. Importantly, these are accompanied by neurological and visual improvement. Although used as second-line therapy upon relapse, ongoing studies are comparing the long-term benefits of MEK inhibitors when compared to chemotherapy. Future directions may include early treatment of infants with NF1 in an effort to prevent the development of brain tumors and plexiform neurofibromas.

2.2.3 Replication Repair Deficiency Syndromes (Constitutional Mismatch Repair Deficiency (CMMRD) Syndrome, Lynch Syndrome, Polymerase Proofreading-Associated Polyposis (PPAP))

CMMRD is a rare familial cancer predisposition syndrome that has a unique clinical phenotype. We present this syndrome after NF1 since they share some clinical characteristics resulting in misdiagnosis and inappropriate management. Distinction between the two is critical. With increased awareness, brain tumors are now observed in other replication repair-deficient syndromes such as Lynch and PPAP syndromes.

In 1997, the distinction of Turcot syndrome into two distinct entities, known today as the brain tumor-polyposis syndrome (BTPS) types 1 and 2, was suggested [38]. Currently, BTPS-1 is termed Lynch syndrome and is a result of germline heterozygous mutations in one of the mismatch repair (MMR) genes [39]. BTPS-2 is currently termed familial adenomatous polyposis (FAP) syndrome and is associated with a different set of cancers (see below). Tumors of Lynch syndrome patients are mainly colorectal and less commonly urogenital and most commonly occur in early to mid-adulthood.

Although individuals with Lynch syndrome have no clinical features on physical examination, individuals with germline homozygous or compound heterozygosity for the MMR genes (CMMRD) present with café au lait spots and other cutaneous pigmentation anomalies, overlapping with NF1and tuberous sclerosis, but having different spectra of associated malignancies. Other features include developmental venous anomalies, pilomatricomas, agenesis of the corpus callosum, and mild immunodeficiency with decreased levels of immunoglobulins IgG2/IgG4 and IgA, among others. Children with CMMRD are usually affected in the first two decades

of life with hematological malignancies (most commonly T-cell lymphomas), malignant brain tumors, and gastrointestinal cancers, with a wide variety of cancers being reported over the past few years.

Genetics: In humans, germline mutations are reported in *MLH1*, *MSH2*, *MSH6*, and *PMS2*. These mismatch repair genes are critical in repairing single-base-pair mismatches and misalignments [40]. In addition, germline deletions of the *EPCAM* gene, located just upstream of *MSH2*, result in hypermethylation of the *MSH2* promoter in epithelial tissues and MSH2 deficiency [41]. Upon loss of one of these genes (which do not appear to be functionally redundant), high mutation rates are observed including in cancers which are described as having a "hypermutator phenotype" (>10 mutations/megabase) [3]. CMMRD is inherited in an autosomal recessive fashion and is found mostly in consanguineous families. CMMRD individuals may actually have NF1 which is thought to be caused by "secondary" early or germline mutations in the *NF1* gene as a part of the hypermutator phenotype [42]. CMMRD tumors may acquire secondary somatic mutations in the polymerase proofreading genes, polymerase ε (*POLE1*), and polymerase δ (*POLD1*), resulting in an "ultra-hypermutated phenotype" (>100 mutations/megabase). Recent reports suggest that primary germline mutations in *POLE/POLD1*, especially exonuclease domain mutations, can also lead to brain tumors in young children, with many of the clinical features overlapping with CMMRD [43, 44]. Tumors in Lynch syndrome can likewise acquire secondary somatic mutations leading to a hypermutated phenotype, similar to CMMRD [45]. They may biologically behave similarly due to complete loss of the DNA replication repair pathways and are now termed as replication repair-deficient (RRD) tumors.

Brain tumors: High-grade gliomas are the most common type of tumor observed in these individuals usually in the second decade of life. RRD gliomas are characterized by extensive intra-tumoral heterogeneity, poor patient survival, resistance to temozolomide, and heterogeneous but encouraging response to PD-1 immune checkpoint inhibition. Some children present with low-grade gliomas, but these tend to transform to high-grade tumors (secondary glioblastoma), which is otherwise rare in children [46]. Medulloblastoma and other tumors with embryonal morphology have also been reported. Experience from our international consortium suggests inferior survival with conventional chemo-radiation approaches for these "embryonal" tumors. Diagnosis of synchronous and metachronous tumors of different histology and molecular profiles is reported (Fig. 2.2) [47].

Diagnosis: Any patient with glioma, T-cell lymphoma, and either café au lait spots, consanguinity, or family history of colon cancer should be screened for all of the four mismatch repair genes. Similarly, high index of suspicion should be raised for "NF1" patients with malignant gliomas and consanguinity. Testing for microsatellite instability, which is diagnostic for MMR in Lynch syndrome, is not sensitive for CMMRD. Immunohistochemistry (IHC) revealing loss of the corresponding MMR protein in both normal and malignant cells is highly concordant with a diagnosis of CMMRD. Because normal cells are usually positively stained in Lynch tumors and are negative in CMMRD, this simple tool can distinguish between the two syndromes. IHC on normal tissue like skin fibroblasts can diagnose germline

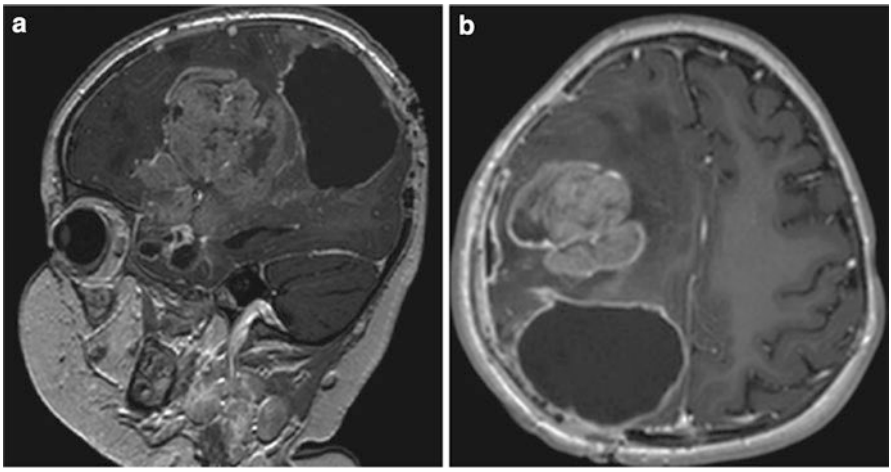

Fig. 2.2 Bifocal glioblastoma in a CMMRD patient. Two separate lesions (**a**. saggital, and **b**. axial views) uncovered in an infant with CMMRD. Molecular and genetic analysis confirmed two different glioblastomas and not metastatic disease. *CMMRD* constitutional mismatch repair deficiency

deficiency in patients without tumors. Genetic testing for specific mutations, functional assays on patient lymphocytes, and a high tumor mutational burden aid confirmation of diagnosis.

Management: Individuals with Lynch syndrome benefit from a strict surveillance protocol (www.NCCN.org) and from preventive colectomy. Therefore, such a diagnosis may benefit parents and other family members. Likewise, it has been demonstrated by both the International RRD Consortium and the European groups that surveillance protocols result in encouraging long-term survival benefits in CMMRD [48]. The goal of surveillance is to identify asymptomatic tumors at early stages when amenable to resection. For high-grade gliomas, resistance to temozolomide, while retaining sensitivity to CCNU, is reported. Radiation does not increase the risk of chromothripsis-driven tumors. All CMMRD cancers are hypermutant, and immune checkpoint inhibitors have shown encouraging results in patients with recurrent CMMRD cancers [49]. There are several reports of better response of MMR tumors to specific agents including retinoic acid [50]. Long-term daily use of acetylsalicylic acid (aspirin) has been shown to reduce cancer risk in individuals with Lynch syndrome but needs to be systematically explored in CMMRD, especially in view of the risk of bleeding with undiagnosed brain tumors [51].

2.2.3.1 Brain Tumor-Polyposis Syndrome 2 (BTPS-2; Familial Adenomatous Polyposis, FAP)

Familial adenomatous polyposis (FAP) is an autosomal dominant cancer predisposition syndrome. It is highly penetrant but may have variable phenotypic expression linked to mutations in specific regions of the relatively large *FAP* gene. It

predisposes to pre-cancerous colorectal, gastric, and duodenal polyps, thyroid neoplasms, hepatoblastoma, pancreatic carcinoma, adrenal tumors, osteoma, fibromas, pilomatrixomas, desmoid tumors, as well as epidermal cysts, dental anomalies, and congenital hypertrophy of the retinal pigment epithelium [52]. A decade prior to Turcot's first description, Crail reported the first association of a patient with colorectal carcinoma and medulloblastoma [53]. Medulloblastoma is the only brain tumor observed in children with this syndrome.

Genetics: FAP is caused by germline heterozygous mutations in the *adenomatous polyposis coli* (*APC)* gene [54]. *APC* is located on chromosome 5q21–5q22 and is a major regulator of the *WNT* pathway which plays a paramount role in controlling development, stem cell viability, and proliferation. Hyperactivation of the *WNT* pathway is reported in 10–15% of medulloblastomas. Around 85–90% of WNT-medulloblastoma harbor somatic activating mutations in exon-3 of *CTNNB1*, which encodes for β-catenin. Majority (6–8%) of the remainder harbor loss-of-function mutations of the tumor suppressor gene *APC* and are associated with FAP, which can upregulate β-catenin via different mechanisms.

Clinical implications: Being mutually exclusive, testing for *APC* mutations in the germline is currently recommended for all WNT-medulloblastoma lacking *CTNNB1* mutations [13]. Although the risk of medulloblastoma development in patients with FAP is considered higher than that of the general population [39], surveillance is not recommended routinely for this tumor. However, patients with WNT-medulloblastoma and underlying FAP should undergo gastrointestinal cancer surveillance. These patients and/or their family members display numerous (>100) small colonic polyps with later onset of malignant transformation to adenocarcinoma. Indeed, patients have been reported with simultaneous diagnoses of medulloblastoma and colonic adenocarcinoma. Although the association between FAP and medulloblastoma has been known for over 20 years, the prognosis of these patients has been indeterminant. A recent report from the French cooperative group suggested that despite excellent survival for FAP-associated WNT-medulloblastoma, there is a high risk of second neoplasms, many of which may have been related to the treatment of the medulloblastoma (radiation and/or surgery) [55]. This supports consideration for treatment de-escalation for FAP-associated WNT-medulloblastoma, akin to approaches being adopted for sporadic WNT-medulloblastoma [56].

2.2.4 Fanconi Anemia and Homologous Recombination Repair Deficiency-Related Cancer Predisposition Syndromes

Fanconi anemia (FA) is a rare autosomal recessive disorder of DNA repair, with characteristically variable clinical expression, including various congenital malformations (in about 60% of individuals), and bone marrow failure states, and/or

myeloid dysplasias or leukemias developing in most individuals. In addition, a variety of solid tumors have long been recognized to develop at increased frequency, particularly liver adenomas (in association with prior androgenic steroid use for the bone marrow failure), and gastrointestinal and gynecological carcinomas, with a median age at diagnosis of about 29 years. The median age for onset of the leukemias is 14 years. It has been estimated that, by theoretically removing the competing risks of marrow failure and leukemias, individuals with FA have an estimated cumulative probability of developing a solid tumor of 76% by the age of 45 years [57].

Although brain tumors in Fanconi patients had been reported in the past [58, 59], the occurrence of medulloblastoma [60] and glioma [61] in association with specific germline mutations has not been suggested until recently.

Molecular genetics: FA is a genetically heterogeneous disorder associated with biallelic mutations of at least 14 genes [62]. Individuals in the FA complementation group *FANCD-1* are estimated to represent no more than 3% of all individuals with FA, and it is this group in whom biallelic mutations with *BRCA2* are found. *BRCA2* mutations are well known to be associated with familial predisposition to breast and ovarian cancer. Brain tumors have also been reported in such families, commonly medulloblastoma and rarely glioblastoma [63, 64]. These individuals may present a more severe phenotype with early onset of cancer. In particular, the cumulative probability of developing a brain tumor (almost always medulloblastoma) could be as high as 85% in the first decade of life [60]. In a multicentric analysis for germline predisposition for >1000 medulloblastoma, Waszak et al. reported the prevalence of germline *BRCA2* mutations in 1% (median age, 3.6 years) and *PALB2* in 0.5% of all medulloblastoma (median age, 5.3 years) [13]. Heterozygous mutations in *BRCA2* and *PALB2* were associated with medulloblastoma with a homologous recombination repair deficiency like mutational signature. Children with compound heterozygous mutations of *BRCA2* exclusively developed SHH medulloblastoma and had a worse 5-year progression-free survival (25%) as compared to heterozygous germline *BRCA2* carriers with medulloblastoma (100%), which could belong to Group 3/4. Germline *PALB2* mutations could be associated with SHH, Group 3 or 4 medulloblastoma, but were all heterozygous, and not associated with Fanconi anemia.

Management: The rare individuals who develop medulloblastoma in the setting of FA do so at a very early age, often before a diagnosis of FA has been made. Individuals with FA undergoing treatment for cancer are well recognized to be highly sensitive to both irradiation and chemotherapy (especially alkylator-based)-associated toxicities [65]. Thus, for any early-onset pediatric brain tumor with cutaneous, skeletal, or neurological abnormalities consistent with a diagnosis of FA, or in case of severe unexpected toxicity from chemotherapy, genetic counseling and testing are recommended. Relevant family history of known associated cancers, or tumor sequencing revealing a characteristic mutational signature profile suggestive of homologous recombination deficiency, may prompt consideration for germline analysis for *BRCA2* and *PALB2* mutations in medulloblastoma. However clinical implications of heterozygous mutations and the role of PARP inhibitors, if any, need to be clarified in larger studies.

2.2.5 *Gorlin Syndrome (Basal Cell Nevus Syndrome, BCNS)*

BCNS is an autosomal dominant condition associated with multiple phenotypic anomalies (coarse facial appearance, macrocephaly, hypertelorism) and predisposition to benign and malignant tumors (basal cell carcinomas and medulloblastoma). The association of multiple nevoid basal cell "epithelioma," jaw cysts, and bifid ribs was first reported in 1960 [66].

Medulloblastoma: The first report of a brain tumor, medulloblastoma, in association with this syndrome [67] was in 1963. The development of medulloblastoma in the setting of BCNS occurs earlier than in the sporadic setting. Most patients are younger than 2 years of age, and all tumors have desmoplastic-nodular histology, often demonstrating extensive nodularity (MBEN). They belong to the SHH subgroup and specifically subtypes SHHβ and SHHγ (Fig. 2.3) [68]. These tumors usually have favorable outcomes without radiation therapy or aggressive resection [69].

Meningioma: Several reports have documented the development of intracranial meningiomas in patients with BCNS who had received craniospinal irradiation. The association between BCNS and the development of meningiomas in adulthood is still controversial, but this tumor was reported in previously non-irradiated patients [70].

Genetics: The gene responsible for NBCCS is *PTCH1* which is located on chromosome 9q22.3 [71]. *PTCH1* encodes a protein that is a major suppressor of the sonic hedgehog (SHH) pathway by direct inhibition of Smo. Disruption of *PTCH1* leads to constitutive activation of the pathway and induction of *Gli* target genes and

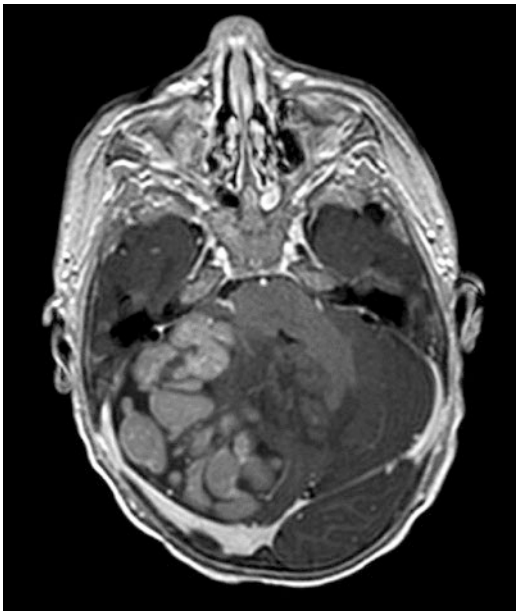

Fig. 2.3 Medulloblastoma in a child with Gorlin syndrome**.** Large right cerebellar mass with multiple nodular components, suggestive of medulloblastoma with extreme nodularity. These tumors have desmoplastic nodular histology, belong to the SHH subgroup, and are exquisitely chemo-sensitive

cell proliferation and survival. SHH is involved in neural development and midline segregation, which can explain some of the lesions in NBCCS. Alternatively, germline mutations in *Sufu*, which is a direct inhibitor of *Gli*, have been reported in familial and sporadic medulloblastoma [72] in up to 50% of desmoplastic tumors [73]. The risk of developing medulloblastoma with germline *PTCH1* mutation is <2%, while this is much higher with those with germline *SUFU* mutation. Inferior survival with higher risk of local relapses has been reported for medulloblastoma having germline *SUFU* mutation (5-year progression-free survival and overall survival rates were 42% and 66%) [74]. However another study reported no significant difference in survival between *SUFU* and *PTCH1* (5-year progression-free survival, 56%; overall survival, 85%) [13]. Interestingly, germline G protein-coupled receptor 161 (*GPR161*) gene mutations were recently reported in up to 5% of infant medulloblastoma and can have an overlapping phenotype with Gorlin syndrome, with additional risk of gastrointestinal neoplasms as young adults [75].

Management: All infants and young children <3 years of age with SHH medulloblastoma need to be screened for germline *PTCH1* and *SUFU* mutations, as this may be the first manifestation of the syndrome. Individuals with the clinical manifestations of BCNS or a family history of basal cell carcinomas or other manifestations of the syndrome should be screened as well. However family history is usually rare (9%) and clinical stigmata detectable in ~67% [13]. Radiotherapy is associated with a higher rate of basal cell carcinoma development within the irradiated fields [76] in almost all patients and should be avoided. The surveillance guidelines have been tailored to the type of mutation [77]. Dermatological surveillance, echocardiogram for cardiac fibromas, and ultrasound for ovarian fibromas are recommended for all, with additional focus on detecting jaw cysts for germline *PTCH1* carriers and regular MRI for medulloblastoma screening in *SUFU* carriers. Because medulloblastoma is the most life-threatening tumor of childhood Gorlin syndrome, and in these individuals usually present by age 2, consideration of very early genetic diagnosis among family members (infants) is recommended. The recent finding of novel SHH inhibitors currently developed for medulloblastoma [78] may offer targeted therapies for individuals with BCNS and possible primary prevention for their tumors.

2.3 Tumor-Specific Cancer Predisposition Syndromes

These syndromes may also include other cancers. However, there is a specific brain tumor type which is pathognomonic of the syndrome, and germline mutations of the corresponding gene are common in this specific tumor entity. Such a tumor diagnosis requires genetic counseling regardless of other manifestations of the syndrome.

2.3.1 Tuberous Sclerosis

Tuberous sclerosis (TSC) is an autosomal dominant multi-system condition affecting both children and adults [79]. Tumors outside the CNS arising in these patients are generally slow growing and include cardiac rhabdomyoma, renal angiomyolipoma, and pulmonary lymphangioleiomyomatosis. Although these lesions are termed benign by pathologists, they can cause significant morbidity and even mortality by causing severe organ dysfunction. Additionally, individuals with TS can rarely develop malignant renal cell carcinomas.

The only CNS tumor seen in patients with TSC is subependymal giant cell astrocytoma (SEGA). This tumor develops in some 5–15% of TSC patients, usually in the first two decades of life, and occurs only rarely in individuals without TSC and then only in older adults. These intraventricular tumors, usually in close proximity to the foramen of Monroe, are histologically benign (considered WHO grade I) but can nevertheless lead to significant morbidity and mortality, due to development of intracranial hypertension from obstruction of cerebrospinal fluid (CSF) flow at the foramen, as well as due to subependymal invasion into eloquent brain parenchyma.

Genetics: Linkage analysis enabled the discovery of two genes responsible for the TSC syndrome. These are *TSC1*, also known as *hamartin*, located on chromosome 9q34 [80], and *TSC2* or *tuberin* on chromosome 16p13. These genes exert their tumor suppressor activity by inhibition of *Rheb*, which is the major activator of *mTOR*. The *AKT/mTOR* pathway is one of the major pathways in carcinogenesis [81].

Management: Brain MRI scans should be obtained at least annually during childhood and adolescence, when the risk for SEGA development is greatest [82]. TSC is a prototype of biological discoveries leading to novel targeted therapies, which may change the spectrum of a disease. The term *mTOR* stems from "mammalian target of rapamycin"; rapamycin can inhibit *mTOR*, directly bypassing *TSC1* and *TSC2* dysfunction. This knowledge has resulted in several clinical trials revealing striking tumor regression of virtually all SEGAs [83–85] (Fig. 2.4) and improvement in pulmonary function for patients with lymphangioleiomyomatosis [86]. Additional evidence suggests that rapamycin analogues can also improve other aspects of the syndrome including neurological status and epileptic activity [87, 88]. Primary prevention and protocols for long-term therapies with *mTOR* inhibitors are currently being developed [89].

2.3.2 Rhabdoid Tumor Predisposition Syndrome (RTPS)

Malignant rhabdoid tumor of the kidney as a pathological entity distinct from Wilms tumor was first described in 1978. The same authors later reported the association of these kidney tumors with second primary "embryonal" tumors in the brain although these brain tumors were reported to be medulloblastomas, a

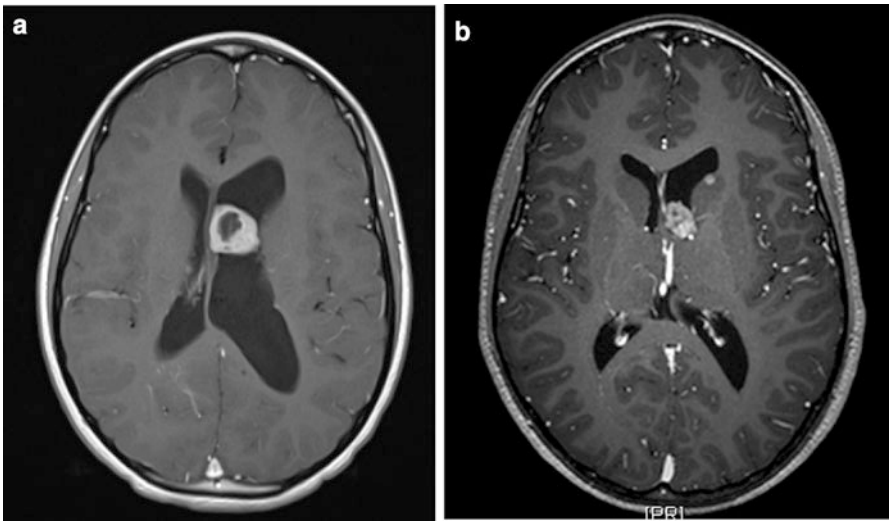

Fig. 2.4 Subependymal giant cell astrocytoma in a patient with tuberous sclerosis. (**a**) SEGA causing mild hydrocephalus in a TS patient. (**b**) Same tumor after 3 months of therapy with oral sirolimus (mTOR inhibitor)

pineoblastoma, a CNS neuroblastoma, and a medullomyoblastoma [90]. Subsequently this distinct pathologic entity was termed atypical teratoid/rhabdoid tumor of infancy and childhood [91] (see Chap. 3).

After that, it became clear that these tumors were very different from other embryonal CNS tumors such as medulloblastomas or ependymoblastomas [92] commonly seen at this age. AT/RT commonly harbors monosomy of chromosome 22 (or 22q deletion). Furthermore, they were recognized to be highly lethal tumors, with virtually all children dying of progressive tumor within 6–12 months of diagnosis [91].

In 1999, both germline (constitutional) and acquired mutations on chromosome 22q11.2 in children with AT/RT were reported [93], and shortly thereafter the term "rhabdoid predisposition syndrome" was coined to define the newly recognized heritable syndrome predisposing to both renal or extra-renal malignant rhabdoid tumors and AT/RTs [94]. By 2008, the entity of "rhabdoid predisposition syndrome" was sufficiently well documented to merit inclusion in the "World Health Organization (WHO) Classification of Tumours of the CNS (fourth Edition)" [95] .

Genetics: The *SMARCB1* or the commonly known *INI1/hSNF5* gene was cloned in 1998 [96] and is located on chromosome 22q11. Heterozygous germline loss-of-function mutations in the gene were first described in 1999 [94]. This facilitated the definition and permitted assessment of the risk of germline mutations in individuals with AT/RT. Germline mutations occur in up to 35% of AT/RT cases and are more commonly diagnosed in younger patients. Patients with combined CNS and extracranial tumors almost invariably harbor the mutation. The exact function of *INI1* is still not completely understood. However, it is thought to be involved in nucleosome

modification [97], and disruption of the gene is involved in a defective spindle checkpoint and chromatin modifications [98]. Patients who carry a germline mutation in *SMARCB1* have rhabdoid tumor predisposition syndrome type 1 (RTPS1; OMIM #609322), whereas those with *SMARCA4* germline mutations have rhabdoid tumor predisposition syndrome type 2 (RTPS2; OMIM #613325). These mutations are inherited in an autosomal dominant manner, with a second "hit," in the form of either a somatic mutation or LOH of the wild-type allele in the tumor. The spectrum of tumors may differ according to the type of mutation. In addition to AT/RT and other rhabdoid tumors, *SMARCB1* carriers can develop schwannomatosis, meningiomas, and malignant peripheral nerve sheath tumors, while *SMARCA4* carriers can develop small cell carcinoma of the ovary, hypercalcemic type. The recent observation that loss of *INI1* leads to activation of the *SHH* pathway is intriguing in terms of potential therapeutic options [99].

Management: Until recently, the prognosis for children diagnosed with AT/RT was considered almost uniformly grave. It remains unclear whether the improved prognosis in older children with AT/RT reflects the ability for tolerance of higher doses of brain irradiation, and thus more uniform administration of such irradiation, or rather reflects a differing biology of these tumors in older children. The prognosis for young children with CNS AT/RT appears to have improved through better molecular diagnosis and classification of the tumor and implementation of aggressive surgical resection of primary tumors followed by intensive chemotherapy [100–102]. Data on outcome specifically for children with AT/RT in the setting of a rhabdoid predisposition syndrome is limited. Children with RTPS may present with synchronous or metachronous tumors. These patients should undergo surveillance screening which includes imaging evaluations of both the abdomen and the brain. Consensus guidelines for surveillance have been published recently [77].

2.3.3 von Hippel-Lindau Syndrome

von Hippel-Lindau (VHL) syndrome is a tumor predisposition syndrome characterized by a variety of CNS and extraneural tumors (see Chap. 5). CNS hemangioblastomas are pathognomonic and necessitate genetic testing. Historically, vascular tumors of the retina were first described in 1879 by Panas and Remy. von Hippel ultimately described these retinal tumors more comprehensively as well as vascular tumors in the viscera (von Hippel E, 1904); their connection with the often fatal cystic vascular tumors of the cerebellum awaited the first report of Lindau in 1926 [103].

The VHL syndrome is inherited as an autosomal dominant disorder with very high penetrance of over 90% by age 65 years. The most common manifestation of the disease is CNS and retinal hemangioblastomas, which occur in 70% and 60% of patients, respectively [104]. However, the age-limiting tumors are renal cell carcinomas that occur in up to 20% of individuals.

CNS hemangioblastomas: These tumors arise, in order of diminishing frequency, in the cerebellum (44–72% of all patients with VHL syndrome), the retina (25–60%), intramedullary spinal cord (13–50%), brainstem (10–25%), supratentorial compartment (<1%), and lumbosacral nerve roots (<1%) [104]. CNS hemangioblastomas arising as single tumors outside of the posterior fossa are rarely sporadic, and multiple hemangioblastomas are virtually pathognomonic for the presence of a *VHL* germline mutation. The mean age of diagnosis of CNS hemangioblastomas is between 30 and 35 years. However, a minority will present in adolescence and as early as 9 years of age. While CNS hemangioblastomas are considered "benign" tumors, prior to the recognition of their association with VHL and the establishment of screening guidelines for early detection, these tumors were associated with significant morbidity and mortality.

Other intracranial tumors: Retinal hemangioblastomas (angiomas) are found in up to 60% of all VHL syndrome patients and are thus the single most common tumors in the VHL syndrome. They are often multifocal and are bilateral in over 50% of individuals. While the mean age at presentation is 25 years, 5% of retinal angiomas present in children less than 10 years of age, even in infancy, and are therefore the first tumors in VHL individuals to present. Endolymphatic sac tumors arise in the endolymphatic duct between the sigmoid sinus and the internal auditory canal, often bilaterally, and are reported in about 11% to 16% of all VHL individuals [105]. The mean age of diagnosis is about 22 years, but these tumors have been reported in patients as young as 12 years of age.

Genetics: The *VHL* gene is located on the short arm of chromosome 3 (3p25–3p 26) and was first identified as the VHL tumor suppressor gene in 1993 [106]. The *VHL*-encoded protein product interacts with other proteins and forms a substrate recognition unit for ubiquitin ligase, which targets the hypoxia-inducible factor (HIF) genes 1 and 2 for degradation. Under normal circumstances, hypoxia results in HIF proteins to activate multiple metabolic and oncogenic pathways in the cell including increased levels of VEGF, PDGF, erythropoietin, and TGF-beta. Abnormal VHL protein results in constitutive activation of HIF and other factors and end result of continuous cell cycle entrance, modulation of cell death [107] and dramatically increased angiogenesis, and tumor formation [108].

Management: Whereas hemangioblastoma is rare in children, all patients diagnosed with this tumor should be screened for germline mutations in *VHL*. De novo mutations are common and represent up to 20% of patients. Since mosaic mutations are reported, multiple hemangioblastomas, several tumors, or family history of tumors compatible with the VHL spectrum can establish the diagnosis even in the absence of a mutation in blood leukocytes. A surveillance protocol has been developed and is commonly applied for these individuals [109]. This protocol is aimed at potentially improving survival but also reducing morbidity from earlier interventions for VHL tumors [110]. However, the association of VHL and renal cell carcinoma resulted in the development of compounds which inhibit HIF hyperactivation of the VEGF pathway in sporadic renal cell carcinoma. These *anti-angiogenic* drugs have been applied successfully in patients with hemangioblastomas [111–113]. Although only preliminary, such targeted therapies may offer long-term tumor control or prevention for

these patients in the future. Recent reports of successful management using pazopanib have generated interest and need to be validated in larger cohorts [114, 115].

2.3.4 Cowden Syndrome (Multiple Hamartoma Syndrome)

Cowden syndrome was first described in 1963 and named after the first reported patient, Rachel Cowden, who had multiple mucocutaneous hamartomatous abnormalities [116]. About 90% of patients who develop Cowden syndrome develop clinical manifestations before 20 years of age, although they may not be diagnosed into the third decade of life. Women have between a 25% and 50% lifetime risk of developing breast cancer as well as an increased risk of developing endometrial cancer, and both men and women have a 10% lifetime risk of developing epithelial thyroid cancer. About 50% of cases of Cowden syndrome are considered to be inherited.

Brain tumors: The recognition that cerebellar dysplastic gangliocytoma (Lhermitte-Duclos disease) might be a manifestation of Cowden syndrome was first reported in 1991 [117]. The cerebellar dysplastic gangliocytoma was first reported by Jean Lhermitte and P. Duclos in 1920. While more commonly seen in adults [118], about 5–10% occur during childhood.

Genetics: Cowden disease is a member of the PTEN hamartoma tumor syndrome (PHTS). This syndrome encompasses four major clinically distinct syndromes associated with germline mutations in the tumor suppressor *PTEN*. These allelic disorders, Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, Proteus syndrome, and Proteus-like syndrome, are associated with unregulated cellular proliferation leading to the formation of hamartomas [119, 120]. Thus far, an increased risk of malignancy has only been documented in Cowden syndrome.

PTEN is located on chromosome 10q23 and is a phosphatase that competes with *PI3K*, a major protein kinase, by reducing *PI3P* levels in cells. Reduction of 3-phosphoinositides decreases activity of kinases downstream of *PI3K* such as *Akt* and *mTOR* and is responsible for its tumor suppressor activity. The *PI3K/AKt/mTOR* pathway is a major oncogenic pathway which regulates cell survival, proliferation, migration, and angiogenesis [121]. Although *PTEN* alterations are a major component of adult gliomagenesis [3], individuals with germline *PTEN* mutations do not have increased susceptibility for these tumors. Cowden syndrome has been associated with a germline mutation of the *PTEN* gene in about 80% of cases with an additional 10% harboring mutations in the *PTEN* promoter region. Interestingly, 9% of Cowden-like cases have been reported to harbor germline *PIK3C* instead of *PTEN* mutations [122].

Management: Overall, the incidence of cerebellar dysplastic gangliocytoma has been estimated to be 15% among patients with Cowden syndrome undergoing magnetic resonance imaging surveillance scans, with additional patients revealing meningiomas (5%) and other vascular malformations in 30% [123]. Therefore, it is recommended that patients with Cowden syndrome undergo annual surveillance

screening with brain MRI. Surveillance guidelines for individuals with Cowden syndrome are available (www.NCCN.org) and should be utilized for family members [121]. Recent observations of reduction in hamartomas for patients with PHTS after treatment with rapamycin [124] and the finding of excess levels of *mTOR* pathway expression in LDD tumor tissue [125] suggest that prevention or treatment of LDD and other neoplasms in individuals with Cowden syndrome is feasible.

2.3.5 Neurofibromatosis Type II (NF2)

NF2 is an autosomal dominant cancer predisposition syndrome characterized by the development of bilateral vestibular schwannomas and schwannomas of other cranial, spinal, and peripheral nerves (see Chap. 4). NF2 individuals can also develop intracranial, spinal, and optic nerve sheath meningiomas and low-grade ependymomas and gliomas of the central nervous system [126, 127]. There are no similarities in the genetic background and clinical manifestations between NF1 and NF2 except for the name, and they should not be confused.

Intra-cranial Tumors: The hallmark characteristic of NF2 is the development of vestibular (acoustic) schwannomas (Fig. 2.5), eventually most commonly bilateral, with a lifetime penetrance of over 95% in NF2 individuals. Although originally considered to be a syndrome of little pediatric relevance, up to 20% of cases will present at less than 15 years of age [128]. The next most common presentations are related to non-vestibular schwannomas (33%), meningiomas (31%), and spinal

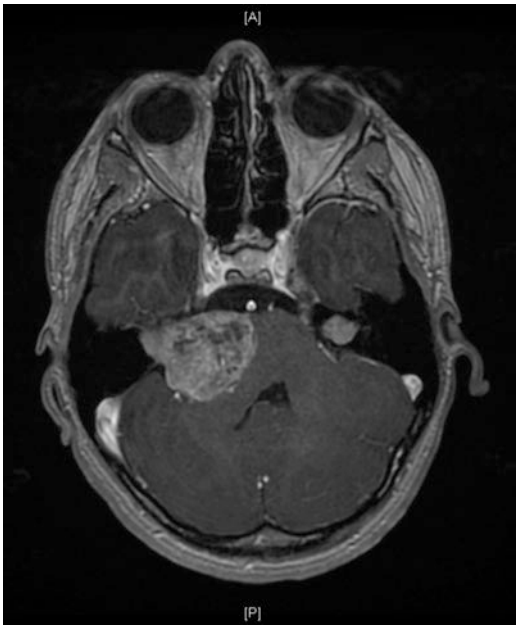

Fig. 2.5 Bilateral acoustic neuromas in a patient with neurofibromatosis type 2

tumors (11.5%); of note, the meningiomas of the brain and spine are frequently multiple. Acoustic schwannomas are usually bilateral in children with NF2 and cause hearing loss and facial nerve palsy. If not resected, they eventually can expand and cause other brainstem-related complications.

Spinal Tumors: The incidence of spinal tumors in patients with NF2 may reach 89% [129]. About one-third of spinal tumors in association with NF2 are intramedullary, most frequently ependymomas and to a lesser extent astrocytomas. Of the extra-medullary tumors, schwannomas are most common, followed by meningiomas, with neurofibromas being very uncommon. These tumors may be asymptomatic and multifocal.

Genetics: The gene responsible for NF2 is *Neurofibromin 2* or *Merlin* located on chromosome 22q12.2 [130, 131]. Interestingly, the exact function of this gene is still not entirely clear. The tumor-suppressive effects of *Merlin* are partially mediated by its membrane organization of proteins, cell-to-cell adhesion, and cytoskeletal architecture. However, more direct nuclear effects have been recently described [16]. There is a high rate of mosaicism in de novo individuals with NF2. As a consequence, the diagnosis of this syndrome often has to be established on clinical criteria alone. Furthermore, transmission, which is autosomal dominant, may be less than 50% in such individuals.

Management: Consensus surveillance guidelines for individuals with NF2 have been published recently [132]. Asymptomatic children carrying the mutation are encouraged to initiate screening from the age of 10 years in order to avoid unnecessary morbidity. Furthermore, recent advances in molecular understanding have facilitated the development of targeted therapies for vestibular schwannomas permitting preservation of hearing for some patients [133]. Other targeted therapies are under development and evaluation [134]. Bevacizumab has been recently reported to be well-tolerated and able to delay hearing loss in children with NF2-related vestibular schwannomas [135, 136].

2.3.6 Other Rare and Emerging Predisposition Conditions in Pediatric Central Nervous System Malignancies

In addition to germline G protein-coupled receptor 161 (GPR161) gene mutations reported with an overlapping Gorlin-like phenotype in SHH medulloblastoma in infants and older children, novel germline *elongator* (*ELP1*) mutations have been recently reported with older children with SHH medulloblastoma. Germline *LZTR1* mutations have been reported in schwannomatosis as well as vestibular schwannomas [137]. Germline *SMARCE1* mutations are reported with clear cell brain and spinal meningiomas [138]. Consensus surveillance guidelines for both these conditions were published recently [132]. Intracranial sarcomas may be associated with germline *DICER1* syndrome and should follow the recommended surveillance guidelines [139, 140]. Familial melanoma astrocytoma is a cancer predisposition syndrome caused by inactivating germline alteration of the *CDKN2A* tumor

suppressor gene. Individuals can develop both melanomas and astrocytomas (predominantly high-grade glioma) and occasionally other nervous system neoplasms including peripheral nerve sheath tumors and meningiomas [141, 142].

2.3.7 Promises and Challenges of Next-Generation Sequencing for Brain Tumor-Related Predisposition Syndromes

With the advances of next-generation sequencing technologies, the drastic drop in sequencing costs, and the increase in diagnostic accuracy, it has become feasible to sequence cancer genomes (and corresponding constitutional DNA) in a growing proportion of cancer patients. This fact will raise new possibilities for the early detection of cancer predisposition without the need of hallmark clinical features or a positive family history of cancer to establish the diagnosis. This could potentially enable enrollment of asymptomatic carriers in surveillance programs and thus diagnose their malignancies early (e.g., in Li-Fraumeni patients who usually have no other clinical symptoms). But it also poses new challenges. For example, surveillance for low-penetrance syndromes will probably result in an excess of unnecessary biopsies. Furthermore, consensus approaches for the management of incidental findings are not in place, and thus ethical issues arise as to how or whether these findings that are not linked to the cancer should be reported back to the patient.

2.3.8 Implications of Molecular Tumor Testing on Genetic Counseling

Most patients are referred to genetic counseling based on a combination of a family history of cancer or other diseases and findings on clinical examination. This approach may change in the near future due to implementation of pathological and genetic tests as routine for tumor diagnosis. These may suggest cancer predisposition in the absence of the above clinical findings. Several examples are worth mentioning. A child diagnosed with SHH-driven medulloblastoma less than 3 years old has a 50% likelihood of having BCNS. AT/RT and choroid plexus carcinomas mutated for *SMARCB1* and *TP53*, respectively, also carry very high rates of germline mutations. Furthermore, many of the children older than 5 years with *TP53* mutant SHH medulloblastoma may be Li-Fraumeni syndrome patients. Since these molecular tests are routinely used now and will be a part of all modern clinical trials, the indications for genetic counseling may change, and the spectrum of tumors and clinical manifestations of some of these syndromes may change as a result.

2.4 Summary

This chapter does not aim to summarize all clinical and molecular aspects of childhood predisposition syndromes associated with brain tumors. Further information is available in other chapters of this book and in the references provided. Furthermore, the syndromes elaborated above are the more common ones and are summarized in Table 2.1. Nevertheless, the burden of cancer predisposition in pediatric neuro-oncology is significant, and knowledge of the diagnosis, management, and appropriate treatment will impact the patient and family members. Importantly, since surveillance protocols have shown survival benefit and novel therapies exist for some specific genetic alterations, individuals with germline mutations in cancer predisposing genes may benefit from early detection and personalized therapies for their cancer which will eventually reduce morbidity and mortality than for sporadic cases.

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Chapter 3
Rhabdoid Tumors

Jaclyn A. Biegel and Jacquelyn J. Roth

Abstract Rhabdoid tumors are rare malignancies that typically present in infants and children. The most common anatomic locations are the central nervous system, where they are referred to as atypical teratoid/rhabdoid tumor (AT/RT), the kidney, and other soft tissues (extra-renal rhabdoid tumor). The vast majority of rhabdoid tumors arise as the result of homozygous inactivation of the *SMARCB1* gene on chromosome 22. Predisposing germline mutations or copy number alterations of *SMARCB1* are present in approximately 25% of patients. Germline and acquired mutations of the *SMARCA4* locus have also been described in probands and family members with rhabdoid tumors. This chapter will address the spectrum of alterations seen in rhabdoid tumors and related malignancies associated with *SMARCB1*, as well as summarize our current and evolving approaches to genetic counseling for this disease.

Keywords Rhabdoid tumor · SMARCB1 · SWI/SNF · DiGeorge syndrome · Schwannomatosis

Rhabdoid tumor is a rare, clinically aggressive, and often fatal malignancy that typically arises in infants and young children. The most common anatomic locations are the central nervous system, referred to as atypical teratoid/rhabdoid tumor (AT/RT), kidney (malignant rhabdoid tumor, MRT or RTK), and soft tissues (extra-renal rhabdoid tumor), including the lung, liver, orbit, neck, skin, and extremities. Rhabdoid tumors account for approximately 1–2% of renal [1] and brain tumors [2] in the

J. A. Biegel (✉)
Department of Pathology and Laboratory Medicine, Children's Hospital Los Angeles, Los Angeles, CA, USA
e-mail: jbiegel@chla.usc.edu

J. J. Roth
Department of Pathology and Laboratory Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA

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pediatric population. There is a slight male predominance [1, 2]. Rhabdoid tumors in all anatomic locations have a peak incidence in the first 3 years of life, and those infants diagnosed in the neonatal period through the first year of life have the worst prognosis [1]. Patients often present with metastatic disease at diagnosis, which impacts therapy and is a negative predictor of outcome. Successful treatment requires surgery and combination chemotherapy regimens, often accompanied by stem cell rescue, with or without radiation therapy [3–11]. Although 5-year survival rates were originally less than 11% [3, 4], limited institution [5] and European collaborative group studies (EU-RHAB) [10] and the first prospective Children's Oncology Group Trial ACNS0333 [9] suggest that short-term survival may approach 40% with the introduction of more intensive regimens. However, the associated morbidity that results from high-dose chemotherapy and radiation, especially in infants with AT/RT, is significant. Novel strategies utilizing direct injection of oncolytic viruses or poly(beta-amino ester) nanoparticles containing herpes simplex virus type I thymidine kinase are in development, based on their effective inhibition of rhabdoid tumor cell proliferation both in vitro and in xenograft studies using RT cell lines [12–14], and may help avoid some of the toxicity associated with chemotherapy and cranial radiation.

Rhabdoid tumor of the kidney was initially described as a rhabdomyosarcomatous variant of Wilms tumor [15], although it is now known that the genetic etiologies for Wilms tumor, rhabdomyosarcoma, and MRT are quite distinct. During the next two decades, histologically similar tumors in the brain and soft tissues were described [16, 17]. The most interesting of these reports was a case series that included seven patients with synchronous embryonal tumors of the brain and MRT [18]. In the first extensive report from the National Wilms Tumor Study Group, 15 of 111 patients with MRT also had a malignant brain tumor, suggesting that they had a genetic cancer predisposition syndrome [19].

Rorke et al. [2] reported the first large series of patients with rhabdoid tumors of the brain, in which they described the complex histology of these tumors. Less than 15% of the tumors presented with pure rhabdoid histology. The rhabdoid cells had large eccentrically placed nuclei with prominent nucleoli and abundant and eosinophilic cytoplasm composed of whorls of intermediate filaments, visible by electron microscopy, which showed positive staining with antibodies to epithelial membrane antigen (EMA) and vimentin (Fig. 3.1). In addition to the rhabdoid component, the vast majority of tumors had areas composed of densely cellular tumor mimicking primitive neuroectodermal tumor (PNET). Epithelial differentiation was normally confined to a few small, gland-like spaces, and a fascicular architecture lent a sarcoma-like or mesenchymal appearance. This combination of malignant histologic components was reminiscent of what is seen in teratomas and led Rorke to coin the name "atypical teratoid tumor" (ATT). The term in use today, AT/RT, exemplifies the finding that tumors may contain a variety of distinct histologic areas, with or without a predominant rhabdoid component. Although the cell of origin has not been confirmed and may be age-dependent, in vitro and in vivo models of rhabdoid tumors have suggested that they arise from pluripotent or neural stem cells [20–22]. In mice, the phenotype is exquisitely sensitive to gestational age and promoter used for inactivating the key driver of rhabdoid tumors, *SMARCB1*.

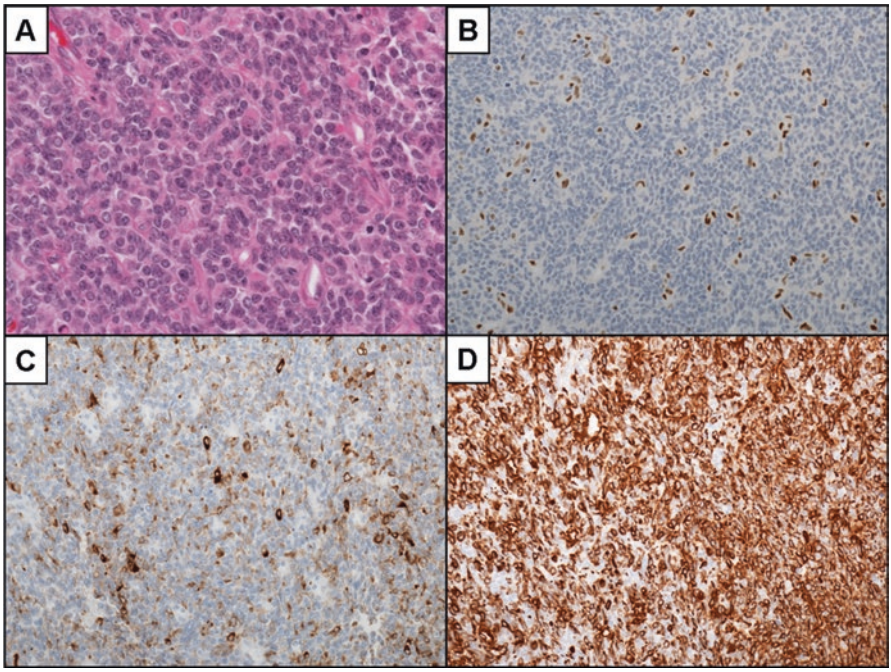

Fig. 3.1 Representative histopathology of AT/RT. (**a**) AT/RT cells demonstrate peripherally placed nuclei, prominent nucleoli, and eosinophilic cytoplasm (H&E, magnification—400×). (**b**) Immunohistochemistry analysis shows loss of SMARCB1 protein expression is seen in AT/RT cells along with positive SMARCB1 staining in the endothelial cells (SMARCB1, magnification—200×). (**c**) Scattered AT/RT cells show immunoreactivity for epithelial membrane antigen (EMA) (EMA, magnification—200×). (**d**) AT/RT cells are strongly immunoreactive for vimentin (vimentin, magnification—200×). (Figure provided by Dr. Mariarita Santi)

Renal and extra-renal soft tissue rhabdoid tumors also demonstrate complex histologic patterns with rhabdoid cells and primitive neuroepithelial, epithelial, spindle, and lymphoid cells [17, 19, 23, 24]. This is especially true for epithelioid sarcomas, which may be difficult to distinguish from extra-renal rhabdoid tumor.

3.1 Identification of *SMARCB1* Alterations in Rhabdoid Tumors

Cytogenetic studies yielded the first clues as to the genetic etiology of rhabdoid tumors. Several case reports and small clinical cohorts of children with MRT, AT/RT, and extra-renal rhabdoid tumors described overlapping deletions in chromosome band 22q11.2, suggesting the presence of a tumor suppressor gene that mapped to this region of the genome. Versteege et al. [25] first reported somatic mutations of the *hSNF5* (*INI1*) gene in MRT and extra-renal rhabdoid tumors,

followed shortly thereafter by the report from Biegel et al. of germline and somatic mutations in *INI1/hSNF5* in patients with rhabdoid tumors of the brain, kidney, and soft tissues [26]. *SMARCB1*, for S*WI/SNF*-related, *m*atrix-associated, *a*ctin-dependent *r*egulator of *c*hromatin, subfamily *B*, member *1*, is now the recommended nomenclature for this gene, replacing the former names *h*uman *s*ucrose *n*on*f*erment-ing gene number *5* (*hSNF5*), *in*tegrase *i*nteractor *1* (*INI1*), and *47*-Kd Brg1/*Brm-a*ssociated *f*actor (*BAF47*).

SMARCB1 is a member of the human *SWI/SNF* complex [27, 28]. The *SWI/SNF* complex acts in an adenosine triphosphate (ATP)-dependent manner to remodel chromatin and both activates and represses gene transcription. In the cancer setting, *SMARCB1* functions as a tumor suppressor gene. Tumors arise as a consequence of inactivation of both copies of the gene, due to mutations, structural alterations, and mitotic recombination events leading to loss of heterozygosity. The homozygous inactivation of the genomic locus results in loss of nuclear expression of the SMARCB1 protein, which facilitated the development of a specific immunohisto-chemistry (IHC) assay for SMARCB1 (Fig. 3.1) [29]. The IHC assay has had unsur-passed clinical utility for distinguishing rhabdoid and related SMARCB1-associated tumors from other brain tumors, including medulloblastoma, primitive neuroecto-dermal tumor (PNET), and choroid plexus carcinoma (CPC) [29], as well as a vari-ety of tumors in soft tissues [30]. Rhabdoid tumors may also arise in the setting of a previously benign tumor, in both the brain [31, 32] and peripheral nervous system [33, 34], following acquisition of a *SMARCB1* mutation and/or deletion. The loss of expression of SMARCB1 by IHC clearly distinguishes the rhabdoid areas from the other (less malignant) components of the tumor. Although *SMARCB1* is the pre-dominant gene altered in rhabdoid tumors, approximately 2–3% of tumors with rhabdoid histology retain expression of the SMARCB1 protein by IHC and do not display inactivating mutations in the gene. Less than 1% of these tumors arise as a consequence of germline and/or somatic alterations of a second rhabdoid tumor predisposition locus, *SMARCA4* [35, 36]. The majority of germline *SMARCA4* mutations have been associated with small cell carcinoma of the ovary hypercalce-mic type (SCCOHT) [37, 38], leading to the hypothesis that SCCOHT is a variant of extra-renal rhabdoid tumor with a different cell of origin. It is likely that the remaining rhabdoid tumors without *SMARCB1* inactivation will also demonstrate alterations in other members of the SWI/SNF or another chromatin-remodeling complex.

A combination of approaches has been used to characterize the spectrum of alter-ations of *SMARCB1* and chromosome 22 in patients with rhabdoid tumors, includ-ing standard cytogenetic analysis and preparation of karyotypes, fluorescence in situ hybridization (FISH), Sanger-based and next-generation sequencing of genomic DNA or cDNA, and single-nucleotide polymorphism (SNP)-based genomic arrays [25, 26, 39–42]. A representative SNP array profile for chromosome 22 is shown in Fig. 3.2, demonstrating a large deletion in the proximal part of 22q and a smaller deletion that includes exons 6–9 of *SMARCB1*. Confirmation of the exon 6 to 9 deletion was performed by multiplex ligation probe amplification (MLPA). MLPA is currently the most sensitive clinical assay used for detecting intragenic deletions

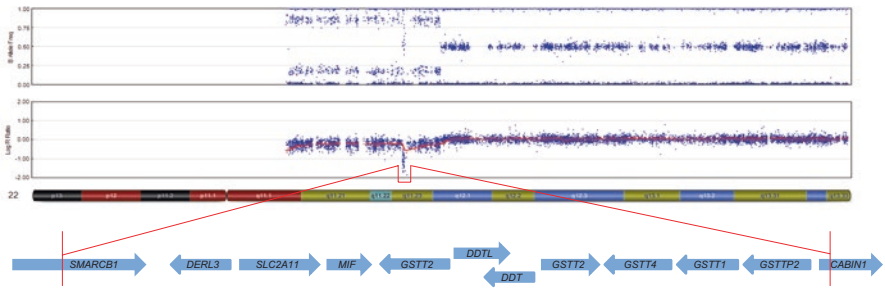

Fig. 3.2 Intragenic homozygous deletion in *SMARCB1* revealed by Illumina (San Diego, CA) Human610-Quad single-nucleotide polymorphism (SNP) array. The upper plot shows the B-allele frequency and the lower plot the intensity (LogR) ratio for chromosome 22 in a single rhabdoid tumor. A deletion of the proximal portion of chromosome 22 from 22q11.1 to 22q12.1 is apparent from the loss of heterozygous alleles and the lower intensity ratio. An overlapping deletion in 22q11.23, demonstrated by the further decrease in the intensity plot, encompasses the genes shown in the blue boxes, resulting in a homozygous deletion of exons 6–9 of *SMARCB1*. Genes are not drawn to scale

or duplications in *SMARCB1* and has revealed a spectrum of single or multi-exon deletions and duplications in *SMARCB1* in both the germline and tumor tissue in patients with rhabdoid tumors from all anatomic locations [42–44].

Despite the fact that rhabdoid tumors are one of the most clinically aggressive tumors in the pediatric population, the tumors are typically diploid with few genetic alterations other than *SMARCB1* and chromosome 22 loss [41, 42]. Genomic inactivation of *SMARCB1* (and related SWI/SNF complex members) may be the only sequence-level event necessary for a tumor to develop. Whole exome and genome sequencing studies of rhabdoid tumors demonstrated mutations or deletions of *SMARCB1* in virtually every tumor, and there were no other consistently mutated or altered genes [41]. Rhabdoid tumors, in fact, exhibit the lowest frequency of mutations overall compared to every other tumor type analyzed to date [45]. While cooperating genetic events in early murine models of rhabdoid tumors implicated a role for *Tp53* and *Smarcb1* [46], or *Rb* and *Smarcb1* [47] in tumor development, the human tumors do not appear to have *TP53* or *RB1* mutations [41, 48].

The distribution of *SMARCB1* and chromosome 22-inactivating mutations, deletions, and copy number neutral loss of heterozygosity (CN-LOH) in 193 sporadic AT/RTs, MRTs, and extra-renal rhabdoid tumors are shown in Table 3.1. In the majority of tumors (45.5%), there is a mutation in one allele, and the second copy of the gene is lost due to either a structural deletion in 22q11.2, monosomy 22, or as a result of an acquired CN-LOH event. Compound heterozygous mutations are infrequent in these patients (4%). Partial deletions and duplications, revealed by SNP array or MLPA, are detected in 15.5% of tumors. Homozygous deletions of exons 1–9 of *SMARCB1* are present in 39% of rhabdoid tumors overall, although there is an unequal distribution with respect to anatomic location. Approximately 25% of AT/RTs, 40% of MRTs, and 70% of extra-renal rhabdoid tumors have homozygous deletions of the entire locus.

Table 3.1 Acquired *SMARCB1* alterations in 193 sporadic rhabdoid tumors

	Allele 2 alteration				
	Mutation	Partial gene deletion/ duplication	Whole gene deletion	CN-LOH	Total
Allele 1 alteration					
Mutation	8 (4%)	1 (0.5%)	52 (27%)	27 (14%)	88 (45.5%)
Partial gene deletion/ duplication	–	5 (3%)	14 (7%)	11 (5.5%)	30 (15.5%)
Whole gene deletion	–	–	75 (39%)	–	75 (39%)
Total	8 (4%)	6 (3.5%)	141 (73%)	38 (19.5%)	193 (100%)

The mutations in sporadic rhabdoid tumors include single base-pair point mutations and insertion/deletion (indel) or frameshift mutations that are predicted to introduce a novel stop codon. The majority of mutations have been proposed to result in nonsense-mediated decay, although this has not formally been proven in most cases. The highest frequency of coding sequence mutations among the sporadic tumors is seen in exon 9. Single base deletions in codons 382 and 383 are somatic in origin and associated exclusively with AT/RT [26, 44]. Mutations in exons 2 and exons 4–7 are frequently observed in MRT and AT/RT. Four specific mutations, c.118C>T, c.157C>T, c.472C>T, and c.601C>T, in exons 2, 2, 4, and 5, respectively, are highly recurrent, although they do not appear to be specific for the brain or kidney [26, 43, 44]. Mutations in exons 1, 3, and 8 are rare. A low frequency of splice site mutations has been documented in sporadic rhabdoid tumors, resulting in loss of an exon in the processed message, similar to that predicted by MLPA. In contrast to the above mentioned nonsense mutations and indels, missense mutations do not appear to lead to inactivation of *SMARCB1* in primary rhabdoid tumors. Interchromosomal balanced translocations result in loss of SMARCB1 in renal medullary carcinomas [49] and could mediate loss of protein expression of SMARCB1 in the small number of rhabdoid tumors with only one documented inactivating deletion or mutation of this locus. Mutations in non-coding regions of the *SMARCB1* locus may also lead to altered splicing in rhabdoid tumors, which may be present in the germline and missed by routine clinical copy number and sequencing-based assays [50].

3.2 Germline Alterations in *SMARCB1* Predispose Individuals to the Development of Rhabdoid Tumor

The initial hypothesis that there was a genetic predisposition to rhabdoid tumor was based on reports of individuals who presented with a synchronous brain and kidney tumor or with bilateral renal tumors [18, 19]. The histology of the two tumors was distinct, and they did not appear to be metastases. Lynch et al. [51] reported one family in which two sisters developed paravertebral tumors, but the classic presentation of multi-generation families with rhabdoid tumors was not observed. This was likely due to the fatal nature of these malignancies, which was confirmed once *SMARCB1* was identified as the primary rhabdoid tumor predisposition locus [26]. In fact, germline mutations, deletions, or intragenic duplications of *SMARCB1* are most often de novo [43, 44]. Early reports of patients with inherited *SMARCB1* mutations highlighted families with two or more affected first-degree relatives who inherited a mutation from an unaffected parent [40, 52–54]. There was reduced penetrance for the germline mutations of *SMARCB1* and rhabdoid tumor in these families, and as of yet estimates of cancer risk in carriers have not been established. Sevenet et al. [53] described two families with multiple affected siblings and presumed gonadal mosaicism in one of the parents, which has subsequently been confirmed in additional families [44, 55]. Plon et al. [40] also reported one patient with an apparently de novo, germline mosaic, single-exon deletion in *SMARCB1*, indicating that sensitive detection methods are required to effectively rule out the presence of a predisposing germline alteration in affected individuals.

Twenty-five to 35% of newly diagnosed patients with rhabdoid tumor, and almost all children with two primary tumors, have a germline alteration of *SMARCB1* that predisposed them to cancer [43, 44, 56, 57]. These children are much more likely to be diagnosed in the first year of life than patients with sporadic malignancies (Fig. 3.4). In our patient cohort, the median age at diagnosis for the patients with germline *SMARCB1* alterations was 0.5 years (range, 1 day to 5 years) compared to a median age at diagnosis of 1.5 years (range, 1 day to 32 years) for patients with sporadic tumors (Fig. 3.3).

The spectrum of germline mutations, deletions, and duplications from patients with rhabdoid tumors generally reflects that seen in sporadic tumors (Table 3.2 and Fig. 3.4). Approximately 20% of the germline alterations are deletions in chromosome band 22q11.2 that include all of *SMARCB1*, whereas 25% of the patients have a partial deletion or duplication involving one to five exons of the gene. The remaining patients have a variety of truncating mutations due to single base point mutations or indels leading to a frameshift. Splice site mutations are the least common type of mutations observed in children who first present with a rhabdoid tumor [58].

Three specific mutations, c.118C>T in exon 2, c.157C>T in exon 2, and c.472C>T in exon 4, are the most frequently detected germline mutations [26, 43, 44]. With the exception of the two exon 9 frameshift mutations described below, the same mutations predispose carriers to AT/RT, MRT, and to a lesser extent extrarenal tumors, even in the same patient. In contrast, the two most common mutations

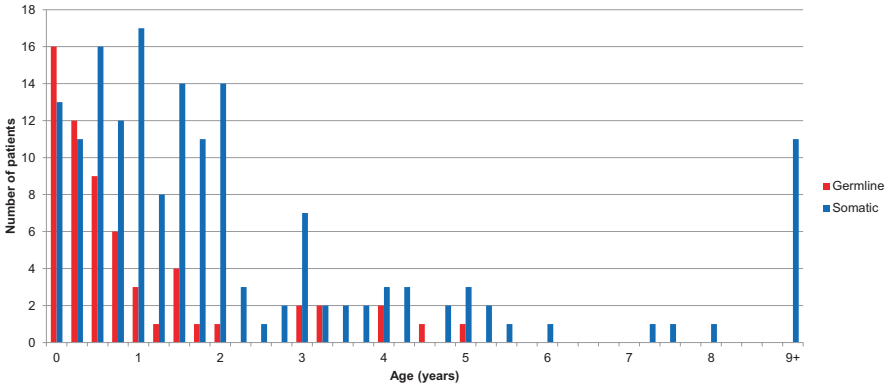

Fig. 3.3 Distribution of the age at diagnosis for 61 rhabdoid tumor patients with predisposing germline alterations of *SMARCB1* and 164 patients with sporadic tumors

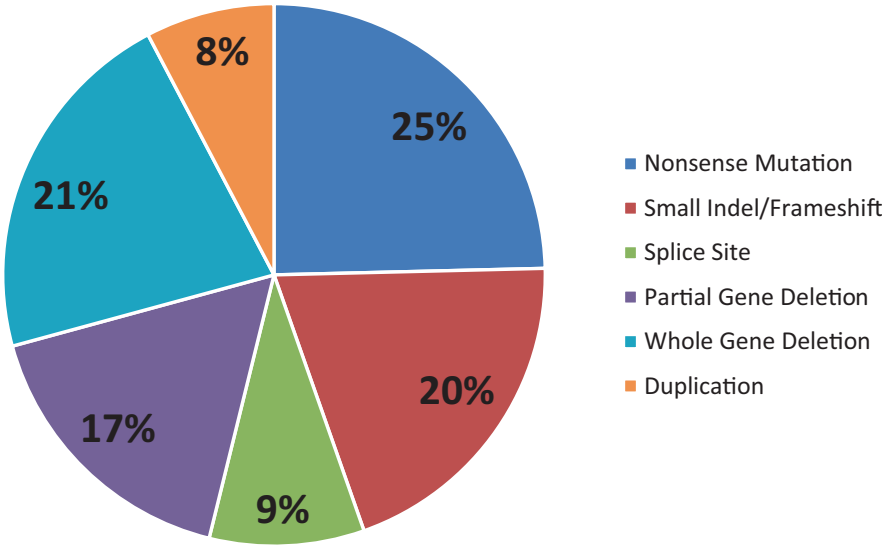

Fig. 3.4 Proportion of germline mutations and gene-level copy number alterations in *SMARCB1* in patients with rhabdoid tumors

in AT/RT, the single base deletions c.1144del and c.1148del (exon 9), have not been detected as a predisposing mutation in the blood from these patients [58]. These two frameshift mutations are not predicted to be subject to nonsense-mediated decay and theoretically would result in the addition of 100 amino acids to the protein. Similar to the other rhabdoid tumors with coding sequence alterations, there is no expression of the protein by immunohistochemistry in AT/RTs with these two exon 9 deletions [29]. It is possible that this mutation functions as a dominant negative mutation during early development, which is an area for future research.

Table 3.2 Germline and acquired *SMARCB1* alterations in 65 patients predisposed to developing rhabdoid tumors

	Somatic alteration				
	Mutation	Partial gene deletion/ duplication	Whole gene deletion	CN-LOH	Total
Germline alteration					
Mutation	2 (3%)	1 (1.5%)	20 (31%)	12 (18%)	35 (54%)
Partial gene deletion/ duplication	2 (3%)	1 (1.5%)	6 (9%)	7 (11%)	16 (24.5%)
Whole gene deletion	7 (11%)	–	5 (8%)	2 (3%)	14 (21.5%)
Total	11 (17%)	2 (3%)	31 (48%)	21 (32%)	65 (100%)

The distribution of the second inactivating events in tumors from the patients with germline *SMARCB1* alterations is shown in Table 3.2. The most frequent second hit is a large 22q deletion or monosomy 22 or a CN-LOH-generating event that unmasks the mutation or deletion on the remaining allele.

The most common chromosomal deletion syndrome in the general population is the 22q11.2 deletion associated with DiGeorge and Velocardiofacial syndrome (DGS/VCFS) [59]. Patients with this genomic disorder have a constellation of abnormalities, including cleft palate, immune deficiency, abnormal ears, heart defects, learning differences, and an increased risk for schizophrenia [59]. The majority of patients with classic DGS/VCFS have deletions mediated by low copy repeats A to D in chromosome band 22q11.2 [59–61]. More distal deletions, which typically include *BCR* (breakpoint cluster region), are associated with a less severe and more variable phenotype [62–66]. Those patients with deletions that extend to low copy repeat G are deleted for *SMARCB1* and thus are at increased risk for rhabdoid tumor [39, 44, 67–71]. With the adoption of high-resolution genomic arrays as the first-tier test for patients with a suspected genomic disorder [72], the identification of infants and young children with distal 22q11.2 deletions that include *SMARCB1* as an incidental finding is becoming more common. This will likely increase as whole-genome copy number analysis and sequencing are utilized in the prenatal setting. Genetics professionals will need to counsel these families about the risk for malignancies in these patients as well as a future risk for development of schwannoma.

Patients with other constitutional structural abnormalities of chromosome 22, including ring chromosomes [73] or deletions of distal 22q13 [74], also appear to be at increased risk for development of rhabdoid tumors. The unstable nature of these structurally altered chromosomes is likely related to the increased risk for monosomy 22, a frequent somatic event in rhabdoid tumors. In contrast, cancers in individuals with the supernumerary derivative chromosome 22 derived from the t(11;22)(q23;q11.2) have only rarely been reported [75, 76].

Rare patients have also been reported with a rhabdoid tumor and another genetic disorder, including Beckwith-Wiedemann syndrome [77], neurofibromatosis type 1 (NF1) [78], epidermolysis bullosa [79], and Goldenhar syndrome [67]. Modena et al. [80] also reported a patient with a germline *SMARCB1* mutation and mosaic Klinefelter syndrome. At the present time, the occurrence of the two genetic disorders appears to be coincidental.

In contrast to the known risk, albeit with reduced penetrance, for carriers of *SMARCB1* alterations to develop rhabdoid tumors, there are no established risk factors for the development of sporadic tumors. Heck et al. [81] performed the first population-based epidemiologic analysis as part of an Air Pollution and Childhood Cancer Study in the state of California. Demographic data and pregnancy history were collected from a total of 105 children with rhabdoid tumors and 208,178 controls under 6 years of age. Fathers were more likely to be white and non-Hispanic, and mothers to have completed a higher level of education than controls. Cases were also more likely to have had private health insurance. The significant findings from the study were an association with low birthweight, preterm birth, and late-term delivery. Of interest, twin pregnancies were seen in association with rhabdoid tumor, which has also been noted by Bourdeaut et al. [43], and in a single-institution case series by Nicolaides et al. [82] that included one twin pregnancy born after in vitro fertilization (IVF). Cecen et al. [83] also reported a single case of a rhabdoid tumor in a patient born after IVF, and we have studied an additional three tumors from children conceived by IVF who had de novo germline *SMARCB1* mutations [unpublished data]. Although some studies suggest a small increased risk for cancer with the use of assisted reproductive technologies [84], this remains controversial [85]. While at present there is only anecdotal evidence to suggest that children born after IVF may be at risk for development of rhabdoid tumors, this should be a subject for further research.

Long-term genotype-phenotype studies are necessary in the context of clinical trials to determine the association of germline and acquired mutations and copy number alterations in *SMARCB1* and *SMARCA4* with patient outcome. Although patients with germline alterations in *SMARCB1* typically present at an earlier age than patients with sporadic tumors, it is not yet known whether this accounts for the apparent increased risk for death in the youngest patients [1, 5, 9, 10]. Patients with germline mutations and deletions of *SMARCB1* have an increased risk for development of second primary tumors, and virtually all patients with second tumors die of their disease. Whether this is due to the underlying presence of the mutation in all cells of their body or resistance to current therapies is also unknown.

3.3 Familial Schwannomatosis and SMARCB1

Neurofibromatosis type 2 is associated with alterations of the *NF2* locus in chromosome band 22q12, and patients typically present with bilateral acoustic neuromas or vestibular schwannomas (OMIM#101000). Schwannomatosis is a distinct disorder,

characterized by the presence of multiple schwannomas, which although histologically benign may result in severe morbidity (OMIM#162091). Treatment of schwannomas is challenging, since the goal is to avoid exposure to mutagenic and carcinogenic agents in a patient with a cancer predisposition syndrome [86]. Tumors that undergo malignant transformation, typically to malignant peripheral nerve sheath tumors, require surgery and chemotherapy.

Genetic association studies of patients with familial schwannomatosis initially demonstrated linkage to chromosome 22; however there were no constitutional mutations of the most likely candidate gene, *NF2* [87]. Boyd et al. [88] and Hadfield et al. [89] demonstrated that approximately one third of patients with familial schwannomatosis have germline mutations in the *SMARCB1* gene. Early genotype-phenotype studies suggested that both splice site mutations and mutations in exon 1 were more common in the familial schwannoma cases than in rhabdoid tumors. A link to *NF2* was nonetheless established with published reports of concomitant deletions in *NF2* in the tumors from these patients. As monosomy 22, large 22q11.2–22q12 deletions, and CN-LOH can all result in loss of the wild-type copy of both *SMARCB1* in 22q11.23 and *NF2* in 22q12, the inactivation of either or both of these loci may influence the phenotype and malignant potential of schwannomas in these families [86].

Some of the most interesting families that have been reported to date are those in which the same germline-inactivating mutation of *SMARCB1* results in schwannoma in older individuals and classic AT/RT in the second and/or third generation [44, 55, 90, 91]. We reported one family in which the proband presented with an AT/RT in infancy [44]. She was found to have a characteristic germline c.472C>T *SMARCB1* mutation. Her father and paternal grandmother were subsequently shown to carry the same germline mutation, and each was also reported to have one or more schwannomas that presented in adulthood. Of note, the proband's paternal great-uncle died in childhood from a malignant brain tumor, which in retrospect was likely an AT/RT. Similar families have been reported with single-exon deletions, duplications, and splice site mutations [55, 91].

It is noteworthy that the families that are ascertained due to the occurrence of a child with a rhabdoid tumor often have a history of a relative who died at a young age with one or more tumors [55], but adult members of the family who are mutation carriers are typically unaffected or develop schwannomas. This suggests that there is a window of time during the early years when the risk for a rhabdoid tumor is the greatest and that the risk decreases with age. This is more typical of families with embryonal tumors, such as retinoblastoma or Wilms tumor, as compared to carriers of mutations in more commonly altered tumor suppressor genes such as *BRCA1* or *PTEN*. The majority of adults with rhabdoid tumors appear to have sporadic disease [92] with acquired inactivating *SMARCB1* mutations and deletions not present in matched blood samples. This is consistent with the hypothesis that the cells that are most vulnerable to sustaining *SMARCB1* mutations or deletions decrease with age, leading to a lower incidence of tumors in older individuals. It is also interesting to note that isolated, sporadic schwannomas do not appear to be due to genomic alterations of either *NF2* or *SMARCB1* (reviewed by [86]). Notably,

Voisin et al. [93] recently reported a sellar AT/RT in a 51-year-old mother from a three-generation family with *SMARCB1*-related brain tumors due to a splice site loss of function mutation in exon 7, confirming that lifelong surveillance will be required for carriers of mutations or inactivating copy number alterations in rhabdoid tumor cancer predisposition genes.

3.4 Molecular Subgrouping of AT/RT

Although inactivation of *SMARCB1* and to a lesser extent *SMARCA4* is the hallmark of rhabdoid tumors, at least three molecular subgroups have been defined on the basis of gene expression and DNA methylation profiling of primary AT/RTs [94]. The ATRT-TYR subgroup is characterized by overexpression of the enzyme tyrosinase, and most tumors in this group can be distinguished by IHC to the protein [95]. ATRT-TYR tumors are also characterized by upregulation of the bone morphogenic protein (BMP) pathway, as well as *PDGFRB*, and *OTX2*. The tumors often demonstrate focal loss involving 22q11.2 in one copy of chromosome 22 and mutation of the remaining copy of *SMARCB1*; patients are less than 3 years of age at diagnosis; and tumors are most often located in infratentorial locations. Cribriform neuroectodermal tumors (CRINETs) demonstrate mutations in *SMARCB1* [96–98] and have a similar expression profile to this group of AT/RTs. Patients with CRINET can achieve a response to combination therapeutic strategies and appear to have a somewhat improved prognosis compared to patients with AT/RT [96]. Clinical and molecular studies of additional patients with CRINET as well as the patients with ATRT-TYR will be required to substantiate these early predictions.

The ATRT-SHH subgroup demonstrates overexpression of sonic hedgehog (SHH) and notch pathway members, including *GL12* and *ASCL1*, respectively. Most tumors have compound heterozygous mutations in *SMARCB1* and fewer deletions in 22q11.2. Patients fall into an intermediate age group, with a median age of 20 months. Two subgroups of ATRT-SHH (ATRT-SHH-1 and ATRT-SHH-2) are distinguished by their supratentorial and infratentorial locations, respectively.

The ATRT-MYC subgroup was named based on the increased expression of the *MYC* oncogene, but without amplification of this locus, and overexpression of several *HOXC* gene clusters. The tumors often demonstrate homozygous deletions of *SMARCB1* and fewer point mutations. The median age of patients in this subgroup is the highest of the three subgroups (median 27 months), but there is a broad range. Spinal cord AT/RTs, as well as other extra-cranial rhabdoid tumors, fall into the ATRT-MYC subgroup, although at least half of the AT/RTs are supratentorial in location.

Fruhwald et al. [10] reported that age less than 1 year and a non-TYR subgroup, as determined by DNA methylation profiling, are independent negative prognostic markers of overall survival for patients with AT/RT. Reddy et al. [9] demonstrated a trend for a worse outcome for patients with germline *SMARCB1* mutations and age, but molecular subgroup was not a prognostic factor in the recently reported COG

ACNS0333 randomized clinical trial. Establishing the prognostic significance of molecular subgroup in AT/RT will therefore require large cooperative group clinical trials in which classification according to molecular subgrouping is performed.

3.5 Loss of SMARCB1 Expression in Other Tumors

Although initial studies of childhood tumors suggested that *SMARCB1* inactivation was specific for rhabdoid tumors [29, 30], we now know that a variety of other tumors also demonstrate loss of *SMARCB1*, either at the genomic or protein level [24]. These include peripheral tumors such as epithelioid sarcoma, malignant peripheral nerve sheath tumor, extraskeletal chondrosarcoma, myoepithelial carcinoma, and de-differentiated chordomas. The spectrum of tumors associated with germline mutations and deletions in *SMARCB1* in the same individual or family is also expanding, which has made it challenging for pathologists to rely on SMARCB1 or SMARCA4 (Brg1) IHC analysis for the differential diagnosis of soft tissue tumors and CNS malignancies. Epithelioid sarcomas are typically associated with homozygous deletions of *SMARCB1*, and yet the frequency of mutations of the gene is extremely low [99, 100]. Neither of these findings can be used to distinguish epithelioid sarcoma from extra-renal rhabdoid tumor. Renal medullary carcinoma, often seen in patients with sickle cell disease, is characterized by loss of SMARCB1 expression by IHC [101], due to balanced chromosomal translocations that interrupt the *SMARCB1* locus [49]. A small percentage of rare collecting duct carcinomas of the kidney may also demonstrate reduced or absent expression of SMARCB1 by IHC, although genomic studies have not yet been performed [102].

3.6 The SWI/SNF Complex and Cancer

SMARCB1 codes for a protein that is a component of the SWI/SNF chromatin-remodeling complex [27, 28]. This complex contains approximately 15 subunits, which requires either SMARCA4 (Brg1) or SMARCA2 (Brm) as the core ATPase for its function [28]. SMARCB1 is considered to be a necessary component of the SWI-SNF complex in all cell types; however the composition of the other subunits appears to depend on developmental state and organ system [27, 103–106]. It is likely that this variability contributes to the predilection for tumors to develop in the brain, kidney, and soft tissues and only extremely rarely in the hematopoietic system [107]. A variety of malignancies in both children and adults have now been described in association with mutations or deletions in almost all of the SWI/SNF members, including the loss of *ARID1A* in ovarian, breast, and endometrial cancers and *SMARCA4* in lung tumors and medulloblastoma [108].

The mechanism by which loss of *SMARCB1* drives cancer formation is an active area of research, yet early studies have already led to the development of several

therapeutic approaches to reverse the epigenetic phenotype of rhabdoid tumors, including HDAC and EZH2 inhibitors [109]. Wang et al. [110] demonstrated that loss of SMARCB1 results in altered enhancer targeting by the SWI/NF complex, particularly at genes required for differentiation, while binding of the complex is retained at super-enhancers. Erkek et al. [111] demonstrated genome wide loss of both H3K27ac and H3K27me3 in *SMARCB1*-deficient AT/RT cell lines. The residual SWI/SNF complex (containing SMARCA4) and Polycomb complex (containing EZH2) were co-localized at active genes in AT/RTs, whereas the REST complex was postulated to impede SWI/SNF residual binding at repressed genes, notably neuronal differentiation genes. The interplay of both activating and repressive mechanisms of gene expression appears to restore tumor cells to a state that is similar to embryonic stem cells.

3.7 Genetic Counseling and Screening

Despite the fact that the genetic etiology for rhabdoid tumors has been known for more than 30 years, genetic counseling for individuals who are at risk for malignancy due to an associated *SMARCB1* or *SMARCA4* mutation is not a common practice outside of tertiary care centers, and surveillance guidelines are not firmly established. This is due in part to the fact that there have been few long-term survivors, and it is only recently that late second malignancies in patients treated in infancy have been reported in patients with predisposing germline mutations. Prenatal genetic counseling is further complicated by the recent reports of SWI/SNF mutations, notably *ARID1A*, *ARID1B*, *SMARCA2*, *SMARCE1*, and *SMARCB1*, in patients with genomic disorders such as Coffin-Siris syndrome (CSS) [112–115] or Nicolaides-Barrister syndrome (NBS) [116–118] who do not appear to be at an increased risk for cancer. Most patients with CSS and *SMARCB1* alterations have heterozygous missense mutations, which are distinct from the typical nonsense mutations seen in patients with rhabdoid tumors or the splice site mutations often seen in familial schwannomatosis. Moertel et al. [119] reported one patient with CSS and a missense mutation in exon 9 who developed multiple schwannomas but not rhabdoid tumor. As whole-genome and whole-exome sequencing move into the arena of prenatal testing, the prediction of whether such mutations will result in a genomic disorder or increased risk for malignancy will become extremely challenging.

At the present time, any individual with a germline *SMARCB1* deletion or mutation should be offered baseline ultrasound or magnetic resonance imaging (MRI) studies to rule out a brain or kidney tumor. The Children's Oncology Group Rhabdoid Tumor Working group initially recommended monthly serial abdominal and transcranial ultrasonography and MRI performed every 3 months during the first year of life, with continued surveillance into the second and third year of life [120]. Rapid whole-body MRI in adult carriers of *SMARCB1* mutations has successfully been utilized to identify schwannomas [121]. Current guidelines for screening patients

with *SMARCB1* and *SMARCA4* alterations are based upon the high penetrance of *SMARCB1* mutations and the high likelihood that a germline *SMARCA4* mutation is inherited but with reduced penetrance [56, 122]. These include monthly physical examinations for the first year, baseline brain and spine MRI, and abdominal ultrasound, followed by brain, spine, and abdominal ultrasounds every 3 months for the first year. Nemes et al. [56] recommend brain and spine or whole-body MRI every 3 months until age 4 or 5 years for patients with AT/RT and abdominal or pelvic ultrasounds or whole-body MRI for patients with extra-cranial rhabdoid tumors. Patients should then be followed in a cancer surveillance clinic with twice yearly physical exams and targeting imaging. For patients with *SMARCA4*-related ovarian tumors, patients should have abdominal and pelvic ultrasounds every 6 months.

Unfortunately, given the rapid development and growth of malignant rhabdoid and related tumors, this may not be a sufficiently effective protocol for early detection and treatment. We are aware of at least two infants diagnosed prenatally with familial *SMARCB1* mutations who developed AT/RT during the first year of life, despite clinical surveillance [43]. Furthermore, patients with germline alterations in *SMARCB1* have developed second primary tumors as late as 15 years after successful treatment for a AT/RT [[123, 124], unpublished data].

Parents of probands should be counseled regarding the risk of recurrence in other children as well as potential family members who may be at risk. This should include counseling regarding gonadal mosaicism if the parents of a child with a germline mutation have negative test results from peripheral blood. Although the risk for gonadal mosaicism has not yet been determined, the theoretical risk for inheriting a mutation from a carrier of a *SMARCB1* or *SMARCA4* is 50%. Siblings and unaffected carriers should be offered the same type of surveillance described above, depending on their age.

SMARCB1 was the first tumor suppressor gene in the SWI/SNF chromatin-remodeling complex linked to the development of cancer. Although they are rare in the population, rhabdoid tumors are one of the most clinically aggressive malignancies in the brain, kidney, and soft tissues in children. Identification of *SMARCB1* alterations as the initiating events in tumorigenesis have formed the basis for an entirely new field of cancer biology which has far-reaching implications for both constitutional genetic disorders and tumors associated with alterations in SWI/SNF function.

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Chapter 4
Neurofibromatosis

D. Gareth R. Evans

Abstract Neurofibromatoses are made up of at least three autosomal dominantly inherited disorders, neurofibromatosis type 1 (NF1), neurofibromatosis type 2 (NF2) and schwannomatosis. For many years, these conditions were inextricably linked as part of generalised neurofibromatosis as first delineated by von Recklinghausen. In 1987 the separate localisation of the NF1 gene to chromosome 17q and NF2 (bilateral vestibular schwannoma) to 22q led to a consensus conference at the National Institutes of Health. At this conference the two main neurofibromatoses, NF1 and NF2, were formally separated. More recently, the *SMARCB1 and LZTR1* genes both on 22q have been confirmed as causing a subset of schwannomatosis. The last 28 years have seen a great improvement in understanding the clinical and molecular features of these conditions. Both NF1 and NF2 provide the clinician with often complex management decisions. Childhood presentation of NF2 in particular predicts a severe multi-tumour disease course. Malignancy is rare in NF2 particularly in childhood; however, there are significant risks in NF1. NF1 is associated with a risk of juvenile myelomonocytic leukaemia (JMML), rhabdomyosarcoma and malignant peripheral nerve sheath tumour as well as a substantial risk of noninvasive pilocytic astrocytoma particularly affecting the optic pathway. The malignancy risk in schwannomatosis is not well defined but may include an increased risk of malignant peripheral nerve sheath tumour.

Keywords Neurofibromatosis · Schwannomatosis · NF1 · NF2 · Café au lait macules · Lisch nodules · Plexiform neurofibroma

D. G. R. Evans (✉)
Genomic Medicine, Manchester Academic Health Science Centre, University of Manchester, Manchester University Hospitals NHS Foundation Trust, St Mary's Hospital, Manchester, UK
e-mail: gareth.evans@mft.nhs.uk

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4.1 Introduction

Neurofibromatoses have for most of their known existence been lumped together as a single entity. This was largely due to the highly influential Harvey Cushing describing that bilateral tumours of the "nervus acusticus" was part of von Recklinghausen disease in the early part of the twentieth century [1]. The clinical and genetic distinction between the two conditions was not fully recognised until the last three decades, and NF1 and NF2 were frequently intermingled in prior reports [2]. Gradually in the latter 20 years of the twentieth century, the differences in clinical presentation and genetic cause resulted in the definition of two distinct conditions, NF1, formerly known as von Recklinghausen neurofibromatosis, and NF2 as bilateral acoustic or central neurofibromatosis. The conditions were eventually recognised as separate entities with the localisation of the respective genes to chromosomes 17 and 22 [3, 4]. This was followed by the formal clinical delineation at a US National Institutes of Health (NIH) consensus meeting in 1987 [5]. The gene for NF1 was cloned in 1990 [6] and the NF2 gene in 1993 [7, 8]. Since 1987 there has been no evidence that either classical NF1 or NF2 fulfilling NIH criteria were anything but homogeneous conditions. Thus far there is no evidence of exclusion of classical NF2 (bilateral VS) from the *NF2* locus on 22q [9] or of NF1 from the locus on 17q. There is nonetheless phenotypic overlap, and families with multiple café au lait patches and macrocephaly without neurofibromas or other typical NF1 features may either have a three-base-pair deletion in *NF1* [10] or a *SPRED1* pathogenic variant (PV) [11]. A third type of neurofibromatosis called schwannomatosis is now accepted [12] with clinical and tumour features which overlap with NF2. A separate chromosomal location for the condition was identified in 2003 [13], with a gene causing at least a proportion of schwannomatosis, *SMARCB1* identified in 2009 [14]. In this chapter, I will delineate the clinical, epidemiological and molecular aspects of NF1, NF2 and schwannomatosis and particularly how they manifest in childhood.

4.2 Neurofibromatosis 1 (NF1)

4.2.1 Genetics and Epidemiology

A number of studies have addressed the genetics, prevalence and incidence of NF1. The autosomal dominant inheritance pattern of NF1 was recognised in the early 1900s. Although many cases present as a de novo mutation of the gene and appear as isolated cases, the presence of the disease features in multiple generations and with transmission from male to male confirmed the gene as an autosomal dominant [2]. NF1 has a birth incidence of 1 in 2052–3300 [2, 15–17] and a diagnostic prevalence of 1 in 4088–4950 [15–17]. The highest frequency was reported in an Israeli study of military recruits with a prevalence of around 1 per 1000 [18]; however, this

was based largely on the presence of ≥6 café au lait patches and could represent a founder effect for the three-base-pair deletion in *NF1* or *SPRED1* PVs [10, 11, 16, 19]. Indeed only two-thirds of children with ≥6 café au lait patches and no tumour features of NF1 had an *NF1* PV [19].

4.2.2 Pathology and Pathogenesis

NF1 is characterised by multiple site tumour and other clinical features [2, 20, 21]. Most features especially tumours are caused by inactivation of both copies of the *NF1* gene leading to loss of the NF1 protein (neurofibromin) in the causative cell. This causes loss of tumour suppressor function leading to a high risk of tumours particularly of neural crest origin. Even the common non-tumour features such as café au lait patches are caused by complete inactivation of *NF1*.

4.2.3 Disease Course

NF1 is widely variable in disease course. This variation is frequently great even within families with an identical *NF1* PV. Such predicting disease severity is difficult. Children with early manifestation of multiple tumour disease are likely to have a more severe disease course, and this may be a manifestation of an early loss of the normal copy of the *NF1* gene or due to a germline large inherited deletion of the *NF1* gene itself or of inheriting a pattern of modifier genes that alter the phenotype. Diagnosis of one clinical feature does not usually imply a high-risk of another complication although there are exceptions as optic pathway glioma is associated with a higher risk of symptomatic gliomas occurring elsewhere in the brain [22].

4.2.4 Clinical Manifestations

4.2.4.1 Diagnostic Criteria

The diagnostic criteria for NF1 are shown in Table 4.1 and when used are unlikely to lead to misdiagnosis or confusion. These were originally devised at the 1986 National Institutes of Health (NIH) consensus conference. Patients with segmental neurofibromatosis can fulfil these criteria, and clinicians should note any segmental involvement as this may mean the child has only a partial or "mosaic" form of the disease. Clinicians need to be aware that a subset of individuals and families with multiple cafe au lait patches +/− axillary/inguinal freckling, without other NF1 primary features, have PVs in the *SPRED1* gene: a condition now called Legius syndrome [11, 16, 19]. One study showed that 8% of children with no family history or

Table 4.1 NIH diagnostic criteria for NF1

Two or more must be present
1. Six or more café au lait macules, the greatest diameter of which is more than 5 mm in prepubertal patients and more than 15 mm in postpubertal patients
2. Two or more neurofibromas of any type or one plexiform neurofibroma
3. Axillary or inguinal freckling
4. Optic glioma
5. Two or more Lisch nodules
6. A distinctive osseous lesion such as sphenoid dysplasia or pseudarthrosis
7. A first-degree relative with NF1 according to the preceding criteria

personal tumour features of NF1 who only had pigmentary criteria harboured a *SPRED1* PV [19]. While the criteria have stood the test of time, they are currently being modified by an international group to recognise the overlap with Legius syndrome and incorporate molecular criteria.

4.2.4.2 Disease Features

The disease features make up some of the categories for the diagnostic criteria:

- Café au lait patches.
- Intertriginous freckling.
- Cutaneous neurofibromas.
- Plexiform neurofibromas.
- Lisch nodules.

In childhood café au lait patches are smaller as reflected in the diagnostic criteria, but they become larger and may merge. They typically have a straight rather than ragged border, and they are often described as like the "coast of California" in contrast to the "coast of Maine" seen in McCune-Albright syndrome. They often fade in later life against the generally darker "dirtier"-looking skin and may be less easy to recognise without a Wood's light. They are flat with no associated hair and have no propensity for malignant transformation. Freckling usually occurs in non-sun-exposed skin with the axilla more frequently affected than the groin. Freckling usually appears later than café au lait patches. Neurofibromas on and under the skin are the characteristic feature of NF1. These often start as pink−/purple-raised soft lesions that can then transform into more "wart"-like growths (Fig. 4.1). Plexiform tumours, which represent an early potentially embryonic tumour, are often visible from birth with diffuse involvement of the skin and underlying structures. About 2–3% of patients have unsightly plexiform tumours affecting the head and neck [22]. The overlying skin is often hyperpigmented and loses elasticity; this often leads to a gravity effect of "sagging" of the tumour. Cutaneous tumours usually start as soft purplish-coloured areas on the skin but can evolve into unsightly warty outgrowths. Subcutaneous nodular tumours occur as growths on peripheral nerves,

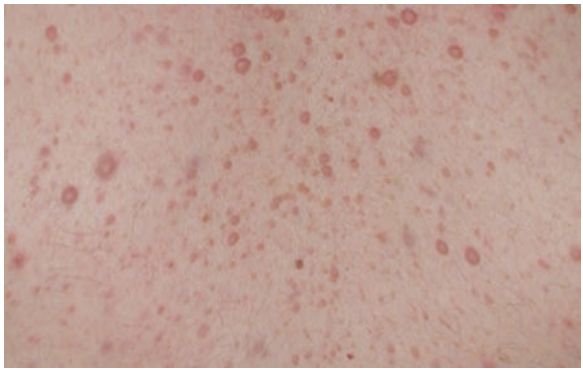

Fig. 4.1 Cutaneous neurofibromas in NF1. Pinkish/purple skin lesions and papillomatous lesions

which are separate from the overlying skin. They may appear as fusiform swellings on more major nerve routes and can be painful to touch. The deeper fusiform subcutaneous and plexiform tumours may undergo malignant change to malignant peripheral nerve sheath tumour (MPNST), although this is uncommon in childhood. Iris Lisch nodules (benign hamartomas) occur early in childhood and usually precede the appearance of cutaneous neurofibromas. They appear as a light brown-orange out-swelling from the latticework of the iris. In contrast to iris nevi which are flat and usually dark brown or black. Ophthalmic slit lamp examination is therefore a useful diagnostic aid in equivocal cases.

4.2.5 History

Clinicians should take a family history for features of NF1 especially relating to the parents of the child. The presence of skin pigmentation (birth marks, Fig. 4.2) from early life is usual with cutaneous lumps occurring around puberty or later. Most NF1 adults will not be in a high-earning profession, and 40% will have had educational problems.

4.2.6 Examination

Full cutaneous examination of the child and their parents is important looking for cutaneous tumours, café au lait, freckling, and possible bony malformations [23]. Slit lamp examination of the irides may also be helpful, and choroidal abnormalities using infrared monochromatic light have been identified as a possible additional method of differentiating NF1 from Legius syndrome [24]. About 5% of children develop xanthogranulomas (small orange-coloured nodules that appear in clusters on the skin) aged 2–5 years, and these were thought to have been associated with an increased risk of juvenile chronic myeloid leukaemia. NF1 patients may also be

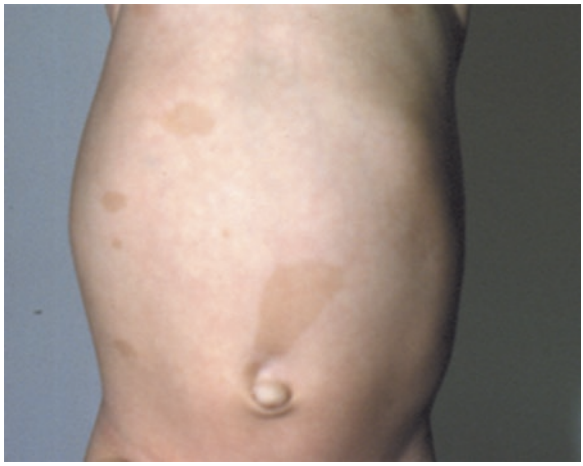

Fig. 4.2 Cafe au lait patches in an infant with NF1

present in childhood with complications from an optic glioma, in particular with visual loss. The tumours themselves are often very benign, and vision may not deteriorate at all from presentation. Other features of optic glioma include precocious puberty with a rapid growth spurt or appearance of secondary sexual characteristics and ocular proptosis. Another rare-presenting feature in the eye is congenital glaucoma in <1%.

4.2.7 Complications

The frequency of disease features and complications is outlined from two UK studies in Table 4.2.

4.2.7.1 CNS Lesions

Large studies where children with NF1 have been screened with MRI or CT scans indicate that around 15% have at least a unilateral optic glioma [25]. It is unclear how many children who have a scan-detected glioma will ever develop symptoms as studies which have not specifically screened with imaging find much lower rates of between 0.7% and 6% [20–22]. Tumours usually present between birth and 6 years of age peaking at around 3–4 years [25, 26], but adult onset of symptoms does occur. Other brain stem gliomas are less frequent affecting around 2–3% of patients but are more frequent in those with optic glioma. About 2% of NF1 patients present with symptoms from spinal tumours that require surgery, but on MRI imaging more than 60% appear to have spinal nerve root involvement. It is not clear why so few spinal tumours present symptomatically, and this is in contrast to NF2 (see later). Other CNS lesions include macrocephaly (45% above 97th centile), aqueduct

Table 4.2 NF1: clinical features, with typical ages at presentation and childhood risk

	Disease feature in percentage (%)	Frequency (paediatric risk) presentation	Age of
Patients in series	**135**	**523**	
Major defining features			
Café au lait spots	>99	98 (98–99)	Birth-puberty
Freckling	67	88 (60–88)	Birth-puberty
Peripheral neurofibromas	>99	60 (20–60)	≥7 years
Lisch nodules	90–95	63 (20–60)	≥3 years
Complications			
Plexiform neurofibromas			
All plexiforms	30	15 (15)	0–18 years
Large lesions of the head and neck	1.2	6 (6)	0–3 years
Limbs/trunk lesions associated with significant skin/bone hypertrophy	5.8	5 (5)	0–5 years
Intellectual handicap			
Severe	0.8	0.5	
Moderate	2.4	2	0–5 years
Minimal/learning difficulties	29.8	35	
Epilepsy			
No known cause	4.4	4.9 (3–5)	
Secondary to disease complications	2.2	0.7	Lifelong
Hypsarrhythmia	1.5	1	0–5
CNS tumors			
Optic glioma	1.5	5 (5–6)	Childhood (usually)
Other CNS tumors	1.5	2.0 (1)	Lifelong
Spinal neurofibromas	1.5	2.0 (0.2)	Lifelong
Aqueduct stenosis	1.5	1.2	Lifelong
Malignancy			
Malignant peripheral nerve sheath tumors	1.5	5 (0.2)	Lifelong
Pelvic rhabdomyosarcoma	1.5	0.2 (0.2)	0–5
Orthopaedic complications			
Scoliosis, requiring surgery	4.4	2.6	0–18
Scoliosis, less severe	5.2	12	
Pseudarthrosis of the tibia and fibula	3.7	2.3	0–5
Vertical scalloping	10.0		Lifelong
Gastrointestinal tumors (neurofibromas and GISTs)	2.2	2.0 (0)	Lifelong
Renal artery stenosis	1.5	0.6	Lifelong
Pheochromocytoma	0.7	0.4 (0.2)	≥10 years
Duodenal carcinoid	1.5	2 (0.1)	≥10 years

(continued)

Table 4.2 (continued)

	Disease feature in percentage (%)	Frequency (paediatric risk) presentation	Age of
Congenital glaucoma	0.7	0.6	0–1
Juvenile xanthogranuloma	0.7	0.6	0–5
Sphenoid wing dysplasia	0	0.6	Congenital
Atypical forms of childhood leukaemia	0	0.2	0–18
Cerebrovascular disease	0	0.6	Childhood (usually)
Glomus tumours in nailbeds	0	0.2 (0.1)	Adults (usually)

stenosis (<1%) and unidentified bright objects (UBOs) on T2-weighted MRI (33%). About 3% of NF1 patients have epilepsy [20, 21].

4.2.7.2 Bony Lesions

Bony abnormalities are frequently congenital and therefore are present from birth. While scoliosis typically advances at puberty, there are often underlying congenital bony abnormalities of the vertebrae. Scoliosis occurs in about 5–9% of cases, with about half requiring surgery. Pseudoarthrosis of the tibia/fibula occurs congenitally in around 1–2%. Sphenoid wing dysplasia and lambdoid suture defects occur in about 1%.

4.2.7.3 Cardiovascular Lesions

Renal artery stenosis (1%) is a much-quoted NF1 complication and is one of the reasons for regular blood pressure checks. However, recently it is becoming clear that vascular events in early adulthood including bleeds and cerebrovascular events are more common than once thought. Indeed the frequency of these events causing death in those aged <30 years was three times the national rate in North America [27]. A male preponderance of early cardiovascular deaths also appears to be the case [28]. Moyamoya disease following radiotherapy is another complication of note particularly for those having received radiotherapy for optic glioma [22].

4.2.7.4 Malignancy

Malignant peripheral nerve sheath tumours (MPNST) are rare tumours occurring in only 1 per million annually in the general population; between 20 and 50% are NF1-associated [29]. NF1 patients have an 8–15% lifetime risk of MPNST [29, 30], but

these are rare in childhood (aged <16 years–~1%), although cumulative risk to age 20 years has been estimated at 2.7% [31]. Nonetheless a rapidly growing deep-seated tumour with pain or neurological deficit needs to be investigated. PET scans are useful in differentiating a benign plexiform tumour from malignant change.

High-grade gliomas occur at increased frequency in NF1 and are often associated with the presence of an optic pathway glioma. Overall they occur in <1% of patients [22, 27], and only 2/45 childhood gliomas in a population-based series were high grade [31]. Juvenile myelomonocytic leukaemia (JMML) is a definitive NF1 complication. It is generally thought to be incurable (autologous bone marrow transplantation seems to offer some promise) but only occurs in about 1 in 300 NF1 patients [28] and was absent from a population series of 524 children followed from birth to 20 years [31].

4.2.7.5 Endocrine Tumours and Other Tumours

Duodenal endocrine (carcinoid) tumours and pheochromocytoma occur in NF1 with a frequency of around 1%, but they are rare in childhood. "Glomus" tumours *can* occur as painful swellings in the nail beds are being increasingly recognised [32]. Gastrointestinal stromal tumours (GIST) were previously called gastrointestinal neurofibromas which occur in around 2% of NF1 patients but again rarely in childhood.

4.2.7.6 Educational Problems

Although a significant proportion of children with NF1 have learning difficulties particularly with reading and/or minimal intellectual handicap, this rarely causes severe handicap and therefore is not usually a presenting feature. Although some studies have shown a large proportion (8–11%) with an IQ < 70 indicating mental handicap population-based studies suggest that less children have moderate or severe handicap (3%) or need special schooling [33]. Learning difficulties improve with extra education, and IQ in adulthood is better. More recently it has been recognised that around 30% of NF1 children are in the autistic spectrum [34].

4.2.8 Investigations

- *In general practice*
- In general terms NF1 patients only need investigations if a complication is suspected. Annual blood pressure checks are advisable, and checking of the skin and back for early scoliosis is important in childhood. MRI scans of the head and

spine should generally only be performed if optic glioma or another CNS tumour is suspected [35].

- *In out-patient (specialist clinic) or community clinic*
- Checks should be more frequent in childhood with at least annual checks of the bone structure (scoliosis, pseudoarthrosis), vision and growth (optic glioma), blood pressure, neurology/intellectual development, and skin. Because of the risk of optic glioma particularly in the first 6 years of life, regular 6–12 monthly visual field checks are suggested [35].

4.2.9 Differential Diagnosis

The main causes for confusion and potential mis-labelling with NF1 are conditions which are associated with pigmentary abnormalities and multiple cutaneous/subcutaneous lumps. If the NIH criteria are used strictly, then misdiagnosis should be extremely unlikely unless only pigmentary criteria are met [19]. Therefore a biopsy of a subcutaneous tumour in multiple lipomatosis or proper assessment of cutaneous pigmentation in conditions such as Fanconi disease, McCune-Albright, congenital mismatch repair deficiency (CMMRD) and LEOPARD syndromes should be conclusive [35]. The more recent cause for confusion has been the recessive forms of inheritance of the Lynch syndrome mismatch repair genes *MSH2*, *MLH1*, *MSH6*, and *PMS2*. Children with homozygous PVs (CMMRD) present with café au lait patches (rarely typical for NF1 or fulfilling NIH criteria) and paediatric malignancy including brain tumours. Cutaneous neurofibromas have also been reported [35]. Perhaps now the greatest chance of misdiagnosis is with Legius syndrome, although again strict application of the NIH criteria will not usually give a problem [19].

4.2.10 Management

NF1 children with little or no problems can be managed by the community paediatrician or a specialised GP. Emphasis is important in childhood on the educational difficulties in NF1.

Hospital management of NF1 may be necessary for disease complications in childhood. Long-term follow-up will be required after optic glioma diagnosis or bony dysplasia. Children with complex NF1 involving a major disease complication should be referred to an NF specialist service for long-term planning.

It is advisable for NF1 patients to be sent to a specialist NF1 clinic aged 15 years so that their transition care can be determined. Most patients will be able to have long-term follow-up by their primary care physician. However, in patients with a

major complication or large tumour, burden follow-up in specialist clinics is advised in adulthood.

4.2.11 Prognosis

Most children and their parents can be reassured that they may never develop a serious complication of the condition. Life expectancy is reduced largely due to MPNST risk in adulthood [27, 28], but this is more likely if there is substantial tumour burden aged 15–20 years and/or the patient has a large NF1 deletion [29]. NF1 children will have a 50% risk of transmission to their offspring, but disease course is usually to variable to predict severity.

4.2.12 Follow-Up

Follow-up should usually be annual in childhood (6 monthly eye checks to 6 years) unless a serious complication [35].

Tests including MRI scans are usually only necessary if the patient is symptomatic. Investigation for rare complications such as leukaemia will depend on presentation and should be suspected when a child has xanthogranulomas although the link is not conclusive [35]. Early breast screening for increased risk of breast cancer [35, 36] in women with NF1 is probably warranted and FDG PET to investigate suspicious lesions for MPNST [35, 37]. Those with large deletions should have a low threshold for PET investigation [38].

4.2.12.1 Predictive Testing

A child may present brought in by concerned parents who are worried; their child may have inherited NF1 from themselves (they have the disorder) or that the child has disease features suggestive of NF1. The requirement for pre-symptomatic testing in NF1 is limited as the condition is usually identifiable in first-degree relatives by about 5–6 years [39]. There are cases for mutation analysis in children with multiple café au lait patches, although the great majority with 6 or more typical patches will have NF1 [19]. The greatest sensitivity of mutation analysis [19, 40] gives the best negative predictive value, and there is some demand for prenatal testing. However, the variableness of disease course even within families [41] makes counselling in this situation problematic.

4.2.13 Treatment

Generally treatment of a malignancy in NF1 is the same as for non-NF1 patients although radiation should be avoided if at all possible [22]. However, a treatment paradigm has been developed with treatment of plexiform tumours with MEK inhibitor drugs which now have FDA approval [42]. These are likely to also find a place in treatment of optic pathway glioma [43] and potentially other tumour manifestations.

4.3 Neurofibromatosis 2

4.3.1 Genetics and Epidemiology

In the UK, a large population-based estimate of birth incidence for NF2 showed that 1 in 28–33,000 people would be born with a PV in the *NF2* gene [16, 44, 45]. Overall diagnostic disease prevalence is less at 1 in 50–56,000 but would be less than 1 in 150,000 in children.

NF2 like NF1 is an autosomal dominant disorder with >50% cases having no family history [16, 45]. Although the transmission rate is 50% in the second generation and beyond, the risk of transmission in an apparently sporadic case of NF2 is less than 50% due to the high rate of mosaicism, which affects >50% of de novo cases [46, 47].

4.3.2 Clinical Manifestations

NF2 is characterised by the development of benign nerve sheath tumours (schwannoma) and meningiomas [48]. The hallmark of NF2 is the development of bilateral vestibular schwannoma (VS) causing deafness and/or tinnitus. Schwannomas also occur on other cranial, spinal, and peripheral nerves. Meningiomas both intracranial (including optic nerve meningiomas) and intraspinal occur more in women than men although boys are more at risk than girls in childhood [49]. There is also a risk of low-grade central nervous system (CNS) malignancies (ependymomas). The Manchester (modified NIH) diagnostic criteria for NF2 are shown in Table 4.3. The original NIH criteria were expanded to include patients with no family history who have multiple schwannomas and/or meningiomas but who have not yet developed bilateral eighth nerve tumours. These criteria have been shown to be more sensitive [50], but a new point-based system has also been developed that may improve sensitivity in childhood [51].

Table 4.3 Diagnostic criteria for NF2 (these include the NIH criteria with **additional criteria**)

Bilateral vestibular schwannomas *or* family history of NF2 *plus*
(1) Unilateral VS *or*
(2) Any two of meningioma, glioma, neurofibroma, schwannoma and posterior subcapsular lenticular opacities
Additional criteria: Unilateral VS *plus* any two of meningioma, glioma, neurofibroma, schwannoma and posterior subcapsular opacities
Or
Multiple meningioma (two or more) *plus* unilateral VS *or* any two of glioma, neurofibroma, schwannoma and cataract

"any two of" refers to individual tumours or cataract, not to tumour types

4.3.3 Presentation

The majority of adults with NF2 present with hearing loss, which is usually unilateral at time of onset. A significant proportion of cases (20–30%) present with an intracranial meningioma, spinal tumour, or cutaneous tumour. Indeed, the first sign of more severe multi-tumour disease in early childhood is often a non-eighth nerve tumour [52]. This has been re-emphasised by a recent study of 53 paediatric meningiomas [53] in which five unsuspected cases of NF2 were uncovered in addition to the nine already known, giving a frequency of 14/40 (42%) of the meningioma series. Adult presentation is therefore often very different to paediatric presentation, in which VS accounts for as little as 15–30% of initial symptoms [52]. There also appears to be a tendency to mononeuropathy, particularly affecting the facial nerve causing a Bell's-like palsy, which does not fully recover years before the detection of a tumour. Some children present with a polio-like illness with wasting of muscle groups in a lower limb, which again does not fully recover. Ophthalmic features are also prominent in NF2. Between 60% and 80% of patients have cataracts, which are usually presenile posterior subcapsular lenticular opacities that rarely require removal. However, cortical wedge opacities may be present from near birth. Optic nerve meningiomas can cause visual loss in the first years of life, and extensive retinal hamartomas can also affect vision. The frequency of various features of NF2 in 4 studies is shown in Table 4.4.

4.3.4 Examination

Cutaneous features are useful in diagnosis; however, skin features in NF2 are much more subtle than in NF1. About 70% of NF2 patients have skin tumours, but only 10% have more than ten skin tumours [46]. The tumours appear to be of at least three different types. The most frequent type is a plaque-like lesion, which is intracutaneous, slightly raised and more pigmented than the surrounding skin, often with excess hair (Fig. 4.3). More deep-seated subcutaneous nodular tumours can often be

Table 4.4 Clinical characteristics of NF2 patients in four clinical studies

	Study			
	Kanter et al.	Evans et al.	Parry et al.	Mautner et al.
	1980	1992	1996	1996
Setting	USA	UK	USA	Germany
Number of cases	73	120	63	48
Number of families	17	75	32	44
Sporadic cases	0	45	17	44
Mean age at onset (years)	20 (of 59)	22	20	17
Onset in childhood	NK	25%	NK	NK
Intracranial meningiomas	18%	45%	49%	58%
Spinal tumours	NA	26%	67%	90%
Skin tumours	32%	68%	67%	64%
>10 skin tumours	NK	10%	NK	NK
Café au lait macules	42%	43%	47%	NK
Cataract	NK	38%	81%	62%
Astrocytoma	NK	4.1%	1.6%	NK
Ependymoma (%)	NK	2.5%	3.2%	6%
Optic sheath meningioma	NK	4.1%	4.8%	8%

NK not known/not assessed

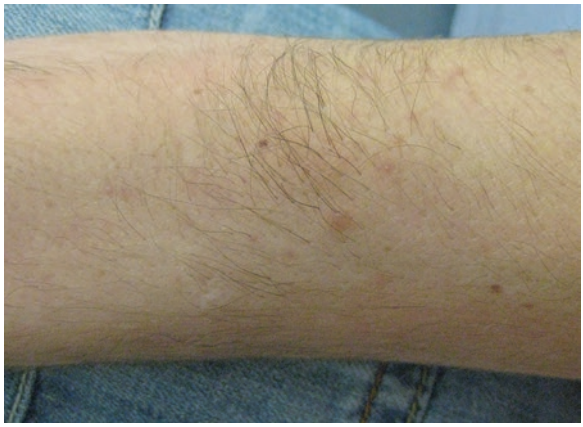

Fig. 4.3 Plaque-like lesions on the arm of a patient with NF2. These are slightly raised, often slightly pigmented lesions that are also frequently hairy

felt, sometimes on major peripheral nerves. These tumours occur as a fusiform swelling of the nerve with thickened nerve palpable on either side (Fig. 4.4). There are also occasional intracutaneous tumours similar to those in NF1. The great majority of these tumours are schwannomas, but occasional definite neurofibromas do occur. Café au lait patches are more common in NF2 than the general population but will only rarely cause confusion with NF1. Ophthalmic examination by a specialised ophthalmologist is important in childhood.

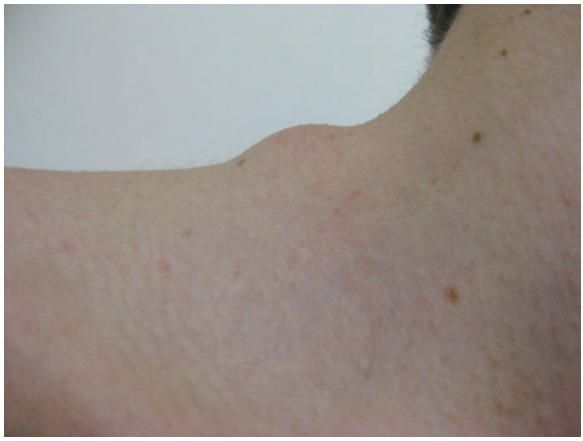

Fig. 4.4 Subcutaneous schwannoma neck angle in a patient with NF2

4.3.5 Radiographic Findings

MRI with gadolinium enhancement (with 1 mm cuts through the internal auditory meatus) will now detect tumours as small as 1–2 mm in diameter on cranial and spinal nerve roots [54]. In children these may already be multifocal at the first investigation [54]. Many of the small spinal tumours will never lead to symptoms. Spinal MRI will detect evidence of spinal tumours in 70–90% of NF2 patients but probably only 50% of children at presentation. There is also increasing recognition of intramedullary tumours, often associated with a syrinx, that predominate in the upper cervical spine and brainstem. On biopsy these tumours are usually low-grade ependymomas. Although these can initially be very worrying for the radiologist or paediatrician, the great majority of these tumours do not progress. Another common finding is schwannomas on other cranial nerves. These occur most commonly on the fifth nerve, but every cranial nerve (bar olfactory and optic) can be affected in NF2 [46]. Nonetheless it is rare for cranial nerve schwannomas other than VS to grow to a size where removal is necessitated. Meningiomas can easily be detected on MRI as enhanced areas on the meninges around the spinal cord, brain or optic nerves (Table 4.4).

There are several groups of individuals who should be considered at risk and investigated further. These groups include those with a family history of NF2, children or young adults presenting with a unilateral VS or meningioma, schwannomas at other sites or retinal hamartoma [55–57]. MRI scanning is vital in their further assessment.

4.3.6 Molecular Genetics

The *NF2* gene was isolated by the simultaneous discovery of constitutional and tumour deletions in a 595 amino acid cell membrane-related gene, which has been called merlin or schwannomin [7, 8]. Large studies have determined genotype/

phenotype correlations with truncating PVs conferring a more severe disease course than missense PVs, splice site mutations or large deletions [58–64]. Position of the PV also correlates with mutations in the 3′ end of the gene (exons 14/15) being associated with fewer meningiomas [49]. Some milder cases have mosaic disease, in which only a proportion of cells contain the mutated *NF2* gene [36, 65–67]. The initiating PV occurs after conception, leading to two separate cell lineages. The proportion of cells affected depends how early in development the mutation occurs. The evidence suggests that up to 58% of NF2 cases without a family history of the disease are mosaic [47], many carrying the mutation in a too small proportion of their cells to be detected from a blood sample [47], and this can be the case even with childhood presentation. Although mosaicism is less in childhood, it still occurs even in a classically affected individual [47, 54, 65]. Mosaicism accounts for the milder disease course in many individuals with unfound mutations, and since only a subset of germ cells will carry the mutation, there is less than a 50% risk of transmitting the disease to their offspring. The risk of transmitting to the next generation will be dependent on the proportion of germinal cells affected. If the mutation is undetectable in blood lymphocytes (only found in tumour cells), then the risk of transmission is low and probably <2% [47, 54]. However, if an offspring has inherited the PV, they will be more severely affected than their parent, since the offspring will carry the mutation in all of their cells.

4.3.7 Management

NF2 presents complex management issues, and a child with NF2 should be managed by a multidisciplinary team consisting of a paediatric neurosurgeon, otolaryngologist, audiologist, ophthalmologist, neuroradiologist and geneticist. An adult neurosurgeon specialising in NF2 is also usually involved. There is clear evidence of reduced mortality benefit [68] with a significantly increased life expectancy for NF2 patients managed at three specialty centres in the UK (RR 0.3, 95% CI 0.12–0.98). This approach was adopted by the highly specialised commissioned service in England and has led to further improved life expectancy with 900 NF2 patients being managed by just four centres [69]. It is important to balance the use of microsurgery and radiation treatment, which can have a role in patients who have particularly aggressive tumours, or who are poor surgical risks, or who refuse surgery. Although radiation treatment has received a great deal of attention and short-term results show good "tumour control", this has to be balanced against longer-term risks such as malignancy especially in childhood [70, 71] and the fact that tumours grow slowly and sometimes not at all for periods of time. Teams experienced in the positioning of brainstem implants can offer partial auditory rehabilitation to those who are deaf, although results are still behind those achievable for cochlear implants. Although the cochlear nerve may be left initially intact after surgery, its blood supply may be damaged; nonetheless a few patients can be rehabilitated successfully with a cochlear implant. Because detection of tumours at an early stage is effective

in improving the clinical management of NF2, pre-symptomatic genetic testing is an integral part of the management of NF2 families. Recently the use of targeted treatments has been highlighted [72–75]. The VegF antibody bevacizumab has been shown to shrink schwannomas and has been used in children [72–75]. However, use in children should be guarded as tumours rebound when treatment is stopped and potential side effects on growth and fertility are still a concern, with renal toxicity another issue [76].

4.3.8 Differential Diagnosis

The main possible diagnostic dilemma with NF2 occurs in isolated patients with multiple non-cranial schwannomas. The *SMARCB1* and more recently *LZTR1* genes have been found to cause this schwannomatosis in a proportion of families [14, 77, 78]. Confusion with NF1 is unlikely since only 1–2% of NF2 patients have six or more café au lait patches and Lisch nodules are extremely rare in NF2, but review of tumour histology is a wise precaution in equivocal cases. The presence of a schwannoma in a patient who does not fulfil NIH criteria for NF1 makes NF1 extremely unlikely, while the presence of multiple neurofibromas makes NF2 very unlikely.

4.3.9 Management and Follow-Up

Management and follow-up should be arranged through a specialised multidisciplinary team [79, 80].

4.4 Schwannomatosis

Schwannomatosis is less common than NF2 and is rare in childhood [45, 80, 81]. Unless a *SMARCB1* or *LZTR1* PV is identified, it is a diagnosis that is made after NF2 has been excluded usually by a combination of cranial imaging to show no evidence of VS, blood *NF2* molecular testing and potentially tumour analysis [45, 80]. Tumours show different *NF2* mutations rather than identical ones which would indicate mosaicism in a patient with negative blood analysis [47, 54]. Nonetheless testing for *SMARCB1* and *LZTR1* is suggested in any child with an isolated schwannoma in addition to NF2 testing as 12% of apparently isolated schwannomas <16 years old have germline PVs in the schwannomatosis genes [56, 80]. Indeed 4% of apparently isolated vestibular schwannomas aged <25 years have germline *LZTR1* PVs [57]. The likelihood of vestibular schwannoma in LZTR1-related schwannomatosis does now cause some overlap with NF2 when using the

Manchester criteria as patients with a unilateral vestibular schwannoma and two or more additional non-intradermal schwannomas are as likely to have a germline constitutional *LZTR1* PV as an *NF2* constitutional PV [82]. This has led to an international group revising the criteria for NF2 and schwannomatosis with a publication date likely in 2021. Risks of childhood schwannoma still appear higher in the less common *SMARCB1* schwannomatosis, and a baseline MRI of the spine and brain is probably justified in children at around puberty [80]. Life expectancy is not usually affected unlike NF2 although pain is a prominent feature [45].

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Chapter 5
Pheochromocytoma and Paraganglioma

Mercedes Robledo, Maria Currás, and Alberto Cascón

Abstract Pheochromocytoma (PCC) and paraganglioma (PGL) are neuroendocrine tumors that originate in the neural crest. While PCCs develop from chromaffin cells in the adrenal medulla, PGLs develop either from paraganglia in the sympathetic nervous system (and are distributed symmetrically along the entire paravertebral axis from the neck to the pelvis, giving rise to thoracic and abdominal/retroperitoneal PGL) or more rarely from parasympathetic paraganglia (giving rise to head and neck PGL and rarely thoracic PGL). PCCs/PGLs have the highest heritability of all human neoplasms being a good example of diseases with underlying genetic heterogeneity. In this regard, at least 40% of PCC/PGL patients carry a germline mutation in 1 of the 19 genes described so far as related to the disease. In addition to the complexity of the genetics of PCC/PGL, we need to consider the role of somatic mutations, which to date have been identified up to 30–35% of tumors. The latter have been observed to occur not only in the same genes involved in heritable susceptibility but also in the new ones, which have thus recently emerged as key players in the sporadic presentation of these diseases. Despite the increasing proportion of patients already explained by germline or somatic genetic defects, there are still patients with clinical indicators of hereditary disease (i.e., family history, multiple tumors, and/or young age of onset) without a molecular diagnosis, which are being actively investigated.

Keywords Pheochromocytoma · Paraganglioma · Pediatric · Genetics · Neuroendocrine tumor · Diagnostic and prognostic markers

M. Robledo (✉) · M. Currás · A. Cascón
Hereditary Endocrine Cancer Group, Spanish National Cancer Research Centre, CNIO, Madrid, Spain
e-mail: mrobledo@cnio.es; mcurras@unav.es; acascon@cnio.es

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5.1 Epidemology

The annual incidence of PCC/PGL in the Spanish population is 2.06 per million (2–8 per million in the USA), which classifies the disease as rare, although results from autopsies suggest a higher incidence [1]. Between 10% and 20% of patients with PCC/PGL are diagnosed during childhood or adolescence [2]. PCC is the most frequently diagnosed endocrine tumor in children [3].

5.2 Clinical Presentation

Both PCC and sympathetic PGL usually manifest as symptoms related to the excessive production of catecholamines [4] and less frequently as symptoms caused by the tumor mass. Parasympathetic PGLs are fundamentally non-functional and therefore almost always present symptoms caused by the tumor mass (Table 5.1).

The average age at presentation of PCC/PGL in pediatrics is 11–13 years, with a male preponderance of 2:1. The classic triad of symptoms (palpitations, sweating, and headache), usually accompanied by hypertension, only occurs in a minority of cases and is particularly unusual in infants. Adrenergic crises can last from seconds to hours, with variable time between crises, from hours to months. They can present spontaneously or result from physical activity (more common in children), changes in posture or an increase in intra-abdominal pressure due to defecation, pregnancy, trauma, or certain diagnostic tests. The clinical presentation is variable, with sustained hypertension seen in 60–90% of pediatric cases, but PCCs/PGLs are the cause of hypertension in only 0.5–2% of pediatric cases [5, 6].

5.3 Tumor Behavior

PCC/PGL diagnosed in infants is more often extra-adrenal (8–43%), multifocal or bilateral (7–53%), malignant (10–47%), and familial (9–50%) [7].

Catecholamine excess, local growth, and metastatic disease all contribute to increased morbidity and mortality in patients with PCC/PGL. Those with sympathetic PCC/PGL have an almost ten times higher incidence of cardiovascular events before their diagnosis. However, mortality is caused mainly by metastatic disease.

The risk of metastasis is greater for sympathetic PGL than for PCC or parasympathetic PGL; however, parasympathetic PGL gives rise to substantial morbidity due to local tumor growth [8]. The following factors are associated with a greater risk of metastasis: carrying a mutation in *SDHB*, young age, persistent postoperative hypertension, large tumor size (>4–5 cm), extra-adrenal or dopamine-producing tumors, and tumors not detected by ^{123}*I-MIBG scintigraphy* [2, 3, 9]. Prognostic data on PCC/PGLs are heterogeneous. Goffredo et al. using data from 18 US

Table 5.1 Clinical presentation [4, 5, 7–10]

PCC and sympathetic PGL	Symptoms in adults: Headache (70–90%), sweating (60–70%), palpitations (50–70%), thoracic and abdominal pain (20–50%), nausea (26–43%), nervousness (35–40%), asthenia (15–40%), blurred vision (3–21%), anxiety and panic attacks (20%), dyspnea (11–19%), heat intolerance (13–15%), dizziness (3–11%), constipation (10%), diarrhea (6%), tremors, weight loss without anorexia, polyuria, and polydipsia Children usually present with headache (81%), sweating (36–68%), weight loss (44%), nausea and vomiting (27–56%), polyuria and polydipsia (25%), constipation (8%), visual disturbances, seizures, and panic attacks
	Signs: – Due to hypersecretion of catecholamines: hypertension (90%), tachycardia (50–70%), fever (66%), pallor (30–60%), vomiting (26–43%), hyperglycemia (42%), flushing (18%), seizures (3–5%), leukocytosis, elevated hematocrit, and hyperglycemia. The onset of an episode of hypertension, tachycardia, and/or arrhythmia related to an invasive diagnostic or therapeutic procedure, anesthesia, the intake of foods rich in tyramine, or the intake of certain drugs should raise the suspicion of PCC/PGL (1). Less frequent in children than in adults are tachycardia, fever, flushing, and hyperglycemia. Dysrhythmia, mood swings, and character changes are rare in children – Due to local compression: Hydroureteronephrosis, renovascular hypertension, etc. Hypertension is the most common sign, yet only 0.05–0.6% of adults and 1–2% of children with hypertension have PCC/PGL – Presentation of hypertension: In adults: Persistent (50%) and paroxysmal (50%); 5–15% of cases have normal blood pressure In children: Persistent in most cases (60–90%) and paroxysmal in only 10%, 20% have normal blood pressure, 80% of pediatric cases present with orthostatic hypotension with or without hypertension Other presentations: "Manic-depressive behavior" of blood pressure: Extreme oscillations over short periods of time, non-*dipper* nocturnal pattern, and hypotension, especially orthostatic hypotension, in adrenaline- or dopamine-secreting tumors
	Medical emergency (occasionally): Hypertensive crisis (often with headache, visual disturbances, and/or seizures), stroke, arrhythmias, ischemic heart disease, cardiomyopathy, congestive heart failure, or pulmonary edema (especially if these become worse on initiating treatment with β-adrenergic blockers), multiorgan failure and even death
Parasympathetic PGL	Unilateral hearing loss, pulsatile tinnitus, dysphonia, cough, pain, Horner syndrome, headache, blurred vision, and dysphagia

registries (time frame 1988–2009) reported a 5-year overall survival rate of 58% for PCCs and 80% for PGLs [10]. More recently, in a retrospective study of 18 European centers, with a follow-up from 1998 to 2010, Hescot et al. reported a global 5-year survival rate of 62% and a median OS of 6.7 years [11].

In the 2017 WHO classification of adrenal tumors, the term benign PCC/PGL was abolished to highlight the fact that all PCC/PGLs should be considered to have metastatic potential. Malignancy occurs in approximately 12% of pediatric/

adolescent patients, which is associated with a 5-year survival rate of 40–95% in adults and 98% in children [12].

5.4 Diagnosis

The diagnosis of PCC and sympathetic PGL is based on biochemical evidence of the levels of catecholamines, while parasympathetic PGL is diagnosed using imaging techniques [13].

The secretion of catecholamines (adrenaline, noradrenaline, dopamine) can be variable and intermittent. In contrast, their conversion to the corresponding metabolites (metanephrine, normetanephrine, methoxytyramine) is continuous and independent of their secretion, which means that the measurement of plasma-free or urine-fractionated metanephrines is more effective in the initial diagnostic screening [14–16].

While determination of metanephrines in plasma has greater specificity and sensitivity (98% and 100%, respectively) than that in urine (both 96%), it requires that the patient meet the following conditions in order to minimize the number of false positives: free of stress, 8–12 h fasting, supine position, and extraction after 20–30 min following insertion of the venous cannula. It is also often much more appropriate in children for which the collection of urine over 24 h can be very challenging. If measured in urine, the level of excreted creatinine should be determined to verify that the sample was collected appropriately [3]. Dietary restrictions are not normally applied, except in the measurement of deconjugated normetanephrine or methoxytyramine in plasma or urine [17].

The range of levels of catecholamines and metanephrines in plasma and urine tends to be higher for hypertense and hospitalized patients compared to normotensive volunteers, for adults compared to children, and for men compared to women. Given that the normal range is usually determined as the 95% central range in a normotensive reference population, defining as positive any result above the upper limit of "normal" may result in an excess of false positives. While age and sex have minimal influence on the normal range for adults, this is not the case for pediatric patients, and so ranges specific for age and sex should be established for biochemical studies carried out in children [16]. In general, when levels more than four times greater than the upper limit of the normal range are observed, the probability of PCC/PGL is high, and further analysis to determine the tumor location is indicated. When increases less than four times the upper limit are observed or when the result is unclear, checks should be carried out for technical errors, inadequate sample extraction, and other clinical conditions that could elevate catecholamines. The result could be confirmed in plasma if originally determined in urine or vice versa [3]. When paroxysmal symptoms are observed in the presence of normal levels, another sample should be collected during or immediately after a paroxysm. It may be informative to carry out a clonidine suppression test, where suppression of $\geq$40% of plasma metanephrines signifies the absence of a tumor [16]. Stimulation tests

have fallen into disuse because of the risks implied and the technical improvements in biochemical tests. Suppression and stimulation tests have not been validated in children and so are generally not recommended in such cases [18].

Chromogranin A is a protein that is co-stored and co-secreted with the amines contained in the secretory granules of neuroendocrine tumors and is therefore a non-specific marker that can be used in clinical follow-up because of its correlation with tumor burden, even in non-secreting tumors [2, 19].

5.5 Determination of Tumor Location

Studies to determine tumor location should only be carried out following biochemical confirmation of diagnosis, except in the case of possible parasympathetic PGL and the follow-up of patients and mutation carriers for which the probability of developing the disease is high [20].

The technique of first choice is computerized tomography (CT) for adults and magnetic resonance imaging (MRI) for children and pregnant women. CT scans allow the visualization of adrenal tumors larger than 1 cm and extra-adrenal tumors larger than 2 cm. The most common image without contrast is that of highly dense ($\geq$10 Hounsfield units) and heterogeneous masses, with an increase in enhancement and a delayed washing following intravenous infusion of contrast. The main drawbacks of this test include exposure to radiation and that tumor identification can be complicated by scaring from prior surgery. MRI is a more expensive technique but has three key advantages: (1) intravenous contrast is not required, (2) it is better than CT in detecting extra-adrenal tumors, and (3) it does not emit ionizing radiation [3, 16].

Head and neck PGLs are highly vascularized tumors that are typically found to be associated with blood vessels and nerve structures. Via CT, the involvement of bone structures can be more clearly defined, and PGL appears as a homogeneous mass with intense contrast enhancement. Using MRI, tumors may appear on T1 sequence surrounded by a matrix of intermediate density, with disperse areas without signal but with intense contrast enhancement, which correspond to the surrounding blood vessels, and on T2 sequence as “salt and pepper” images. In order to establish the involvement of the surrounding vascular structures, it is usually necessary to carry out a selective arteriography, which at the same time allows the embolization of the main artery to reduce hemorrhaging and facilitates surgical resection [21].

Functional tests may be carried out to determine tumor location when other methods fail or require confirmation or in staging in order to identify or rule out metastatic disease or multiple tumors where the location has been established. However, there are no established criteria for their use and no consensus regarding their application prior to surgery. ^{123}I-MIBG scintigraphy is considered the functional test of choice. Somatostatin analogue scintigraphy (Octreoscan®) could be useful for parasympathetic PGL and for those tumors not detected by ^{123}I-MIBG

scintigraphy. In addition, somatostatin analog and MIBG uptake are also predictive factors for targeted internal radiation therapy that can be relevant in the case of progressive metastatic or unresectable disease.

Positron emission tomography (PET) with a radiotracer, combined with CT, is less commonly available but has higher resolution and is more sensitive than *^{123}I-MIBG* scintigraphy to detect extra-adrenal PGL. ^{68}Ga-DOTATATE PET/CT could be used in *SDH*- and *FH*-mutated cases and ^{18}F-DOPA in *VHL*-, *EPAS1*-, *RET*-, *NF1*-, *MAX*-, *TMEM127*-, and *HRAS*-related tumors, both being superior to ^{18}F-FDG PET/CT. At the NIH, the use of ^{68}Ga-DOTATATE PET/CT has extended to pediatric patients with PCC/PGL. Their preliminary results demonstrate the superiority of ^{68}Ga-DOTATATE PET/CT in localization of SDHx-related PCC/PGLs in pediatric population compared to 18F-FDG PET/CT and CT/MR imaging with the exception of abdominal (excluding adrenal and liver) lesions [12, 19, 22].

5.6 Susceptibility to Develop PCC and PGL

These tumors can develop in an apparently sporadic manner or as part of one of several inherited tumor syndromes associated with alterations in distinct genes. Particularly in the latter case, PCC/PGL often presents with other pathologies within a family and even in the same individual. This variable clinical phenotype is testament to the genetic complexity that underlies the development of this disease.

For years PCC/PGL was known as "the 10% tumor," given that 10% were metastatic, 10% hereditary, 10% bilateral, and 10% extra-adrenal. However, the evidence emerging over the last two decades has shown this alias to be erroneous; we now know that more than 30% of patients develop extra-adrenal tumors [23, 24], that the percentage of metastatic cases depends on location of the primary tumor and/or the gene mutated (from approximately 3% for tumors associated with *RET* or *VHL*, up to 70% for those due to mutations in *SDHB*) [6, 25], and that approximately 40% of tumors are due to a germline mutation in one of the known susceptibility genes. In fact, PCC/PGL is the most heritable human tumors, and there are still patients with multiple PGLs and/or bilateral PCC and/or a family history of the disease for which the genetic cause has not been identified. This as-yet unexplained heritability presents a substantial challenge in the quest to understand the tumor biology and correctly genetically classify each patient in order to be able to offer them the most appropriate clinical follow-up.

Since the discovery in 1990 of the first susceptibility gene for PCC, 18 additional genes have been described (Fig. 5.1), highlighting the importance of studies that systematically scan for germline mutations in apparently sporadic cases of PCC/PGL [25].

The 40% of PCC/PGL that is known to be hereditary develops primarily in the context of three familial tumor syndromes: von Hippel-Lindau disease (VHL), multiple endocrine neoplasia type 2 (MEN2), and familial PCC/PGL. Other syndromes

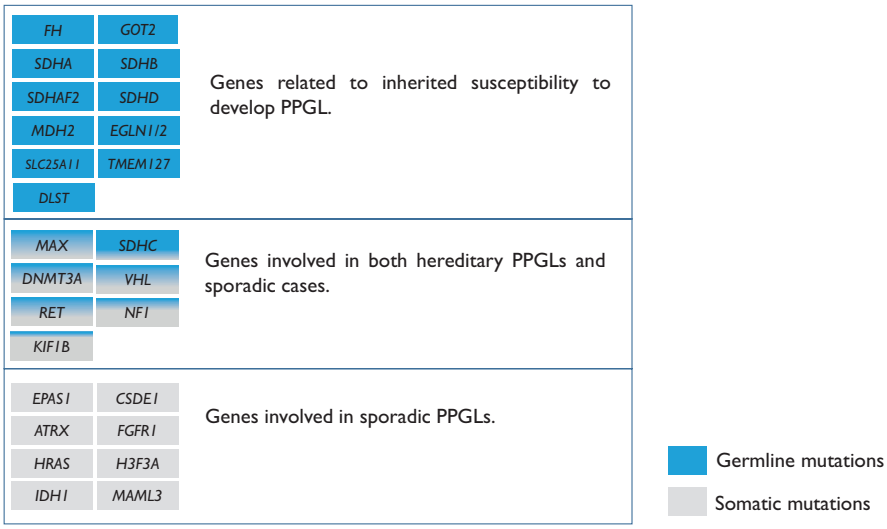

Fig. 5.1 PCC/PGL is a paradigm of genetic heterogeneity. Prior to the discovery by Baysal et al. in 2000 of the first case of PCC caused by one of the genes implicated in mitochondrial respiratory chain complex II, the proportion of cases that were hereditary was 10%, mainly associated with three syndromes, MEN2, VHL, and NF1. Since then, several additional genes have been identified, thereby increasing the proportion of hereditary cases to 40%. There remains a proportion of patients with a personal or family history of PCC/PGL in which no germline mutation has been found in one of the known susceptibility genes. Furthermore, somatic mutations mainly of *RET*, *VHL*, *NF1*, *MAX, EPAS1*, *HRAS,* and *FGFR1* can be detected in an additional 30–35% of the tumors. Overall, the proportion of cases harboring either a germline or a somatic mutation reaches 75%. The proportion of gray indicates the described percentage of somatic mutations for each gene.

are presented in Table 5.2. Patients diagnosed with *neurofibromatosis type I* (NF1) can also develop PCC but do so less frequently. PGLs present almost exclusively as part of familial PCC/PGL.

The proportion of pediatric patients with germline mutations in one of the known susceptibility genes is higher than that found in adults. It has been reported that up to 70–80% of children with PCC are mutation carriers, regardless of their family history [7, 26]. An as-yet unknown proportion of patients with clinical characteristics indicative of hereditary disease (bilateral PCC, multiple PGLs, family history, and/or early-onset disease) does not carry mutations in any of the known genes, suggesting that other loci remain to be discovered (Fig. 5.1); their identification will likely add additional complexity to the genetics underlying the pathogenesis of this disease. This is exemplified by the discovery of post-zygotic somatic alterations in the *EPAS1* (*HIF2A*) gene in patients with multiple PCCs/PGLs, particularly those diagnosed during adolescence [27, 28]. Another example is the case of post-zygotic somatic mutations in the *H3F3A* gene [29], although the prevalence of mutations in this gene has not been addressed so far.

Table 5.2 Genetic and clinical characteristics of genes associated with syndromes related to PCC/PGL development

Gene	Inheritance	Locus	Related syndrome Associated tumors/features
RET	Autosomal dominant	10q11.2	MTC, PHPT, PCC, infrequently PGL
VHL	Autosomal dominant	3p25–26	HB (SNC and retina), ccRCC, neuroendocrine pancreatic tumors, pancreatic cystadenoma, renal cysts, endolymphatic sac tumors, PCC, PGL, etc.
SDHD	Autosomal dominant with maternal imprinting	11q23	Carney-Stratakis syndrome, PGL1, renal cell carcinoma, GIST, pituitary adenoma
SDHC	Autosomal dominant	1q21	Carney-Stratakis syndrome, PGL3, ccRCC, GIST, pituitary adenoma
SDHB	Autosomal dominant	1p35–36.1	Carney-Stratakis syndrome, PGL4, ccRCC, GIST, pituitary adenoma
NF1	Autosomal dominant	17q11.2	von Recklinghausen's disease (0.1–5.7% present PPGL, 3.3–13% based on autopsy studies); Café au lait spots, neurofibromas, axillary and inguinal freckling, Lisch nodules (iris hamartomas), bony abnormalities, optic/CNS gliomas, malignant peripheral nerve sheath tumors, macrocephaly, and cognitive defects
MEN1	Autosomal dominant	11q13	MEN1 syndrome: <1% present PCC Primary hyperparathyroidism, pituitary adenoma, gastroenteropancreatic NET, adrenal cortical tumors, carcinoid tumors, facial angiofibromas, collagenomas, and lipomas
SDHAF2	Autosomal dominant with maternal imprinting	11q13.1	PGL2; H&N PGL> > PCC
TMEM127	Autosomal dominant	2q11.2	PCC, infrequently PGL (head and neck), ccRCC
SDHA	Autosomal dominant	5p15	PGL5, Leigh syndrome (homozygous patients, but no PPGL described), ccRCC, GIST, and pituitary adenoma
MAX	Autosomal dominant by paternal transmission	14q23	PCC (single, bilateral, multiple), up to 20% of patients also develop PGL (thoracic and abdominal), pituitary adenomas
FH	Autosomal dominant	1q43	Reed syndrome or hereditary leiomyomatosis and renal cell cancer (HLRCC), multiple cutaneous and uterine leiomyomatosis (MCUL), cutaneous and uterine leiomyomas, and type 2 papillary renal carcinoma
MDH2	Autosomal dominant	7q11.23	Early-onset severe encephalopathy (homozygous patients, but no PPGL described)

(continued)

Table 5.2 (continued)

Gene	Inheritance	Locus	Related syndrome Associated tumors/features
EGLN1/ PHD2	ND	1q42.1	Hereditary polycythemia, polycythemia
EGLN2/ PHD1	ND	19q13.2	Hereditary polycythemia, polycythemia
EPAS1/ HIF2A	Somatic/ somatic mosaic	2p21	Familial erythrocytosis type 4, Pacak-Zhuang, polycythemia, and somatostatinoma
KIF1B	Autosomal dominant	1p36.22	PCC, neuroblastoma (?), ganglioneuroma (?), leiomyosarcoma (?), lung adenocarcinoma (?), colorectal carcinoma (?)
SLC25A11	Autosomal dominant	17p13.2	PGL6; PGL, PCC
GOT2	Autosomal dominant	16q21	PGL, PCC
DNMT3A	Autosomal dominant	2p23.3	Gain of function mutations: H&N PGL
DLST	Autosomal dominant	14q24.3	PGL7; PGL (multiple) > > PCC

(?) – the association is not clearly demonstated
PCC pheochromocytoma, *PGL* paraganglioma, *H&N* head and neck, *MTC* medullary thyroid carcinoma, *PHPT* primary hyperparathyroidism, *GIST* gastrointestinal stromal tumor, *ccRCC* clear cell renal cell carcinoma, *HB* hemangioblastoma, *ND* no data, although but presumably autosomal dominant

5.7 Syndromic PCC

Some patients develop PCC or PGL as part of a hereditary tumor syndrome; they present with other clinical signs that can point to the gene in which defects are most likely to be involved and therefore help prioritize genetic testing. Such patients have often developed other neoplasms or have a family history indicative of a strong genetic etiology, as is the case for PCC associated with MEN2, VHL or, NF1 and, to a lesser extent, other syndromes such as Carney triad, Carney-Stratakis syndrome, and MEN1. Patients with germline mutations in *RET* more often have been previously diagnosed with medullary thyroid carcinoma (MTC), while those from NF1 families show *cafe au lait spots*. As described in detail below, one exception to this tendency to have particular comorbidities are patients with particular germline mutations in *VHL*, who tend to develop PCC as the sole manifestation of their disease.

5.7.1 MEN2-Associated PCC

MEN2 (OMIM 171400) has an estimated annual incidence of 0.5×10^{-6} and a prevalence of 1 in 30,000. MEN2 follows an autosomal dominant mode of inheritance; causal mutations have variable clinical expression and a penetrance that depends on their transformative capacity. MEN2 patients can develop MTC, PCC, and/or PHPT, the latter resulting from hyperplasia or from parathyroid adenomas. This syndrome is classified into three subtypes: MEN2a, MEN2b, and MTC familiar (MTCf), each defined according to the combination of pathologies developed by the individuals affected. MEN2a patients may develop all three pathologies. In addition, they are more likely to develop a disorder known as "cutaneous lichen amyloidosis," a pruritic skin lesion in the upper area of the back caused by the uncontrolled deposition of amyloid protein between the dermis and epidermis. Rarely they may also develop Hirschsprung disease (HSCR). Patients are classified as MEN2b if they develop, in the absence of parathyroid disease; MTC; PCC; multiple mucocutaneous neuromas involving the lips, tongue, and eyelids; corneal nerve myelination; intestinal ganglioneuromas (*hyperganglionic megacolon*); and *Marfanoid habitus*, including skeletal deformities and hypermobility of joints. Finally, families in which an affected member has developed exclusively MTC or C-cell hyperplasia (CCH) are considered to have the third subtype, MTCf, but only if more than ten members have MTC. An exhaustive clinical follow-up of these families is required to rule out the presence of other tumors characteristic of MEN2, especially in older family members.

Susceptibility to develop MEN2 is caused by germline mutations in the proto-oncogene *RET*. *RET* spans 55 kilobases, includes 21 exons, and encodes a tyrosine kinase receptor that is mainly expressed in cells derived from the neural crest (C cells, parafollicular thyroid cells, and adrenal medulla cells, among others) and in urogenital system precursor cells [30]. Despite its medium size, the genetic testing of *RET* is relatively simple, since the mutations associated with the development of MEN2 affect only a small number of amino acids located on specific exons. Mutations on exons 5, 8, 10, 11, 13, and 14 are related to MEN2a and MTCf, while those on exons 15 and 16 are found in MEN2b patients. The established genotype-phenotype relationships for MEN2 syndrome are based on the classification of individual mutations according to their transforming ability and therefore the expected associated aggressiveness [31]. The impact of *RET* mutation testing on the management of MEN2 patients is without doubt one of the most robust examples of the utility of genetic diagnosis in personalizing clinical follow-up.

Approximately 50% of MEN2 patients develop PCC in their lifetime, and the mean age at diagnosis is 35 years. *RET* mutations are very rarely found in cases diagnosed before age 20 [7, 14, 26], and so *RET* is not a priority in the genetic testing of pediatric patients, although it should still be included in genetic diagnosis algorithms [26]. Between 50% and 80% of tumors are bilateral; they tend to show an adrenergic biochemical phenotype, and a low proportion of tumors are metastatic. A PCC is the first manifestation of MEN2 in only 12–15% of cases, and so

RET explains relatively few cases of non-syndromic disease (around 5%), compared to other syndromes [32] (see Reference [8] for a review of *RET* and MEN2). A recent study by the COMETE consortium reported the presence of somatic mutations in *RET* in a substantial proportion (14%) of sporadic PCC [33]; this finding highlights the importance of working with germline and tumor DNA from the same patients in order to provide a comprehensive genetic diagnosis.

The identification by whole-exome sequencing (WES) of two or more deleterious *RET* mutations in the same patient [34] raises questions regarding their capacity to jointly influence phenotype in MEN2 families. It is likely that the availability of data from large-scale sequencing studies will also shed light on the role of single-nucleotide polymorphisms (SNPs) in modifying phenotype. There are conflicting results from studies focused on the role of SNPs in the development or progression of MTC or PCC in MEN2A patients. Recent studies suggest that p.G691E, or a combination of SNPs, may affect the development of PCC in MEN2A patients [35]. These findings should be confirmed in sufficiently informative families where the co-segregation of these SNPs with the development of PCC is analyzed.

The American Thyroid Association guidelines for PCC surveillance in patients with MEN2 syndromes recommend screening high-risk and moderate-risk patients starting at the age of 11 and 16 years, respectively [36].

5.7.2 VHL

VHL (OMIM 193300) is a hereditary tumor syndrome with a prevalence of 1 in 36,000 and variable clinical manifestation. The penetrance of causal mutations is age-dependent. Affected patients are at higher risk of developing hemangioblastomas (HBs) of the retina and central nervous system (CNS), PCC and/or PGL, clear cell renal cell carcinoma (ccRCC), renal and pancreatic cysts (serous cystadenoma), neuroendocrine pancreas tumors, endolymphatic sac tumors, pancreatic serous cystadenomas, and papillary cystadenomas of the epididymis in men and of the broad ligament in women (Table 5.2) [2, 37, 38].

The diagnosis of VHL is based primarily on the following clinical criteria: patients with a family history and at least one HB of the retina or CNS, PCC, or ccRCC; patients with no family history and at least two HBs or one HB of the CNS; and a visceral injury (other than renal or epididymal cysts, which are both common in the general population). A classification of the disease, including practical information for screening and genetic counseling, has been established and is widely accepted. VHL type 1 families have a low risk of developing PCC but may present with any of the other tumors associated with the disease. Type 2 families develop PCC and HBs and are sub-classified according to the associated low (type 2A) or high (type2B) risk of ccRCC. Finally, type 2C families have PCC as the only clinical sign of the disease.

5.7.2.1 The *VHL* Gene, its Protein (pVHL), and Tumorigenesis

VHL is caused by mutations in *VHL*, a tumor-suppressor gene that has three exons and encodes three gene products: a protein comprising 213 amino acids and two shorter isoforms, one produced by alternative splicing (excluding exon 2) and the other by alternative initiation. While the protein (pVHL) is involved in multiple processes, its best characterized role is in the regulation of the proteasomal degradation of hypoxia-inducible factors (HIFs) [39]. Under normal circumstances, the HIFs mediate the response to hypoxia, augmenting glucose uptake and increasing the expression of angiogenic, metabolic, and growth factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGFB), transforming growth factor (TGF), and erythropoietin (EPO) [39]. Inactivation of pVHL leads to the stabilization of HIF-1α and HIF-2α and therefore to the activation of genes whose transcription depends on these HIFs; this explains the highly vascularized nature of the tumors associated with VHL syndrome [37].

5.7.2.2 PCCs/PGLs Associated with VHL

Approximately 20% of patients with VHL develop PCC or PGL (sympathetic and parasympathetic), although the latter is much less frequent. Tumors show noradrenergic biochemical phenotype, are multifocal or bilateral in 43–45% of cases, and are metastatic in less than 5% [40, 41]. The median age at diagnosis of PCC/PGL is 29 years, which is lower than for other syndromes and particularly relevant for genetic testing since between 12% and 32% of patients with PCC diagnosed during childhood are found to carry a germline mutation in *VHL*. Of note is that PCC (principally) and PGL (occasionally) are the first manifestation for 30–50% of patients with VHL [40]. For these reasons, *VHL* mutation screening is essential in patients diagnosed before age 18. Furthermore, *VHL* has a high mutation rate (20–21%) [42, 43], and so mutation testing of this gene is recommended specifically for apparently sporadic and non-syndromic cases.

The development of VHL-related tumors has been linked to the alteration of specific interactions between pVHL and other proteins with which it forms complexes. The most accepted hypothesis in this regard is that the development of PCC in the context of VHL is associated with a partial retention in the function of pVHL [44]. A hot-spot in *VHL* that is associated with the development of PCC affects residue 167, located in the alpha domain. This domain has the role of interacting with other proteins, so that mutations giving rise to amino acid changes in this region do not result in the loss of function of pVHL. The finding that 23% (7/30) of patients with PCC who carry deleterious germline variants in *VHL*, but have no signs of either VHL or MEN2, have a mutation that affects this residue is consistent with this hypothesis [45].

On the basis of the above findings, it has been proposed that the measurement of the change in pVHL stability could be used as an additional tool to understand the clinical features developed by a VHL patient [44]. Indeed, the use of this tool led to

the identification of an association between ccRCC and missense mutations that significantly alter pVHL stability. A subsequent study classified these mutations as "surface" or "deep," depending on the location of the affected residue in the protein structure, and found a clear difference between them in the associated risk of PCC [38].

Based on the earliest described age at diagnosis, it is recommended that screening be initiated at age 5 years [2]. For more information regarding the genetics of VHL we recommend consultation with the international consensus [46].

5.7.3 Neurofibromatosis Type 1

Neurofibromatosis type 1 (NF1), formerly known as von Recklinghausen disease, is a common hereditary disease with an incidence of 1 per 2500–3300 newborns. It is normally diagnosed in children and is characterized by the appearance of multiple neurofibromas; cafe au lait spots; freckling in the armpits and groin; iris hamartomas (Lisch nodules); bone lesions such as scoliosis, sphenoid dysplasia, or pseudoarthrosis; macrocephaly; learning disorders; cognitive deficits; predisposition to optic and CNS glioma; and leukemia [2, 47]. Although it has been established that NF1 has an autosomal dominant inheritance, close to 50% of patients have de novo mutations, which if they occur post-zygotically can give rise to mosaic phenotypes [48].

5.7.3.1 The *NF1* Gene and its Protein

The gene responsible for NF1, *NF1* (17q11.2), which acts as a tumor suppressor, comprises 60 exons and has one of the highest rates of spontaneous mutation of any gene in the human genome. It encodes the protein neurofibromin, which is expressed primarily in the nervous system and has the role of suppressing cell proliferation by inactivating RAS proteins. Loss-of-function mutations in *NF1* lead to the activation of RAS and the PI3K/AKT/mTOR pathway, which depends on RAS [47].

5.7.3.2 PCCs Associated with NF1

An estimated 0.1–5.7% of NF1 patients develop PCC, although this figure is 3.3–13% based on autopsy studies. NF1-associated PCCs tend to develop at a later age (mean 41 years), can be unilateral or bilateral, are rarely extra-adrenal, and slightly more often metastatic (up to 10%) than those in VHL and MEN2 cases. Recent findings have demonstrated that *NF1* is responsible for a substantial portion of sporadic PCC, with 14–20% of apparently sporadic tumors presenting with somatic mutations in the gene [49–51]. This finding once more highlights the need to study in parallel both normal and tumor tissues from the same patient in order to

carry out a comprehensive genetic diagnosis that is informative for genetic counseling. The prevalence of *NF1* alterations in pediatric PCC is low, and the syndrome is relatively easily identified based on its associated clinical features.

The earliest recorded age at diagnosis of PCC is 7 years, but given the low penetrance of *NF1* mutations, screening is only recommended in cases of hypertension or symptoms suggestive of disease [2]. Other groups have proposed biochemical screening every 3 years starting at age 10 years [52].

5.8 Non-syndromic PCC/PGL

Here we review the genes related to susceptibility to develop PCC or PGL as the only manifestation of the disease. Associations with other tumors have been reported but only in a limited number of patients. We will outline the functions of the SHD and *FH* genes, as well as *TMEM127* and *MAX*, and detail the clinical manifestations associated with mutations in each of these.

5.8.1 Non-syndromic PCC/PGL Associated with Mutations in the SDH *and* FH *Genes*

5.8.1.1 SDH Gene Function

The connection between the SDH genes and the development of neuroendocrine tumors was established in 2000 when germline mutations in *SDHD* were first described in patients with familial PGL [53]. The SDH genes encode Complex II subunits of the mitochondrial respiratory chain, or succinate dehydrogenase (SDH), which plays a key role in both the electron transport chain and the *tricarboxylic* acid cycle. This complex is made up of four subunits: two catalytic (SDHA and SDHB) and two structural (SDHC and SDHD). Heterozygous mutations in the *SDHA*, *SDHB*, *SDHC*, and *SDHD* genes adversely affect the ability of the complex to detect oxygen and cause pseudo-hypoxia, which activates the angiogenic pathway mediated by HIF-1α and VEGF (reviewed in [25]). An additional gene, *SDHAF2*, is also involved in mitochondrial Complex II and in the development of PCC/PGL, confirming the importance of this complex for the disease [54]. SDHAF2 controls the flavination of SDHA, which is critical for the correct functioning of Complex II. The accumulation of succinate caused by mutations in the SDH genes likely causes oncogenesis via the inhibition of prolyl hydroxylases, which are required for the regulation of HIF-1α, mediated by pVHL [55]. This link between mutations in the SDH genes and the HIF-1α pathway is also corroborated by results from tumor expression profiling studies [56]. Mutations in any of the SDH genes, both catalytic and structural, cause defects in the enzymatic activity of the complex, along with the

absence of the protein SDHB [57]; this represents a great advantage in the selection of patients for genetic testing, since if paraffin-embedded tumor material is available, SDHB expression can be determined via immunohistochemistry, and its absence used to indicate the likely involvement of these genes in disease etiology.

5.8.1.2 Mutations in the SHD Genes: Genotype-Phenotype Relationship

Clinical Presentation Associated with Mutations in *SDHD*

The estimated penetrance of germline mutations in *SDHD* (11q23.1) is 86% to age 50 years. Mutations predispose carriers primarily to the development of PGL (84% of cases), although up to 22% also develop thoracic and abdominal PGL and 12–24% PCC, the latter rarely being bilateral [24, 58, 59] (Table 5.2). *SDHD* mutation carriers normally present with multiple PGLs at a mean age of 35 years. While it has been established that *SDHD* defects have an autosomal dominant mode of inheritance, the gene is also subject to maternal imprinting. That is, mutation carriers will only develop the disease if their mutation came from their father; if it came from their mother, they will not be affected, although they will still be able to pass on the mutation to their children (with a probability of 50% for each gestation). This means that the hereditary nature of disease is complete masked in families in which by chance the mutation has been transmitted from generation to generation only from mother to child. In these cases, the disease skips generations, and these can only be identified in genetic counseling centers that collect information from second- and third-degree relatives. Nevertheless, there are reports of families in which the disease has developed in individuals with a germline-mutated maternal chromosome [60, 61]. It has been suggested that the molecular mechanism explaining this involves a loss of the imprinting of the maternal allele, although the probability of this occurring is very low. A key issue in clinical follow-up is the fact that 43.2% of non-proband carriers of a germline mutation in *SDHD* develop malignancies [62]. In the case of pediatric patients, despite the possible lack of family history due to imprinting, it has been suggested that a diagnosis of at least one head or neck PGL is sufficient to justify genetic testing; in fact, 8–16% of patients under age 20 years carries a germline alteration in *SDHD* [7, 26].

In relation to the development of other tumors, it should be noted that there has been some controversy around two variants in *SDHD*, p.H50R and p.G12S. Both were initially reported to be associated with the development of Merkel cell carcinoma and familial CCH and even Cowden-like syndrome. However, they were subsequently classified as SNPs, present in several healthy populations (http://www.lovd.nl/3.0/home), and their associations with the proposed diseases have therefore been ruled out [63]. Screening was recommended starting at 10 years of age for patients carrying a mutation in *SDHD* [64].

Clinical Presentation Associated with Mutations in *SDHB*

An estimated 67% of patients carrying mutations in *SDHB* (1p36.13) develop primarily thoracic and abdominal PGL, 27% develop head and neck PGL, and 17–29% adrenal PCC, which is rarely bilateral [24, 57, 58]. It has been shown that clinical disease penetrance in non-proband *SDHB* mutation carriers is 16%, 22%, and 44% at 50, 60, and 80 years, respectively [62].

Of all the known susceptibility genes for hereditary PCC/PGL, *SDHB* constitutes a paradigm of heterogeneity in and of itself. Mutations in this gene are usually associated with the presence at diagnosis of a single retroperitoneal tumor [65]; this differentiates it from other susceptibility genes that often give rise to bilateral or multiple tumors. However, 23–70% of these single *SDHB* mutation-linked tumors metastasize [6, 24, 66], meaning that it is widely accepted that the identification of a mutation in *SDHB* is a marker of poor prognosis and the need to clinically monitor the patient more closely. An additional issue contributing to the complexity in managing these patients is that while *SDHB* is one of the main genes responsible for pediatric cases of the disease [25, 26, 58], as mentioned above the average penetrance to age 80 years of mutations is only 44% [62]. That is, most mutation carriers never develop PCC/PGL; furthermore, most of those that do have no family history of disease at the time of their diagnosis. This fact, along with the frequent appearance of a single tumor in affected individuals, makes it very difficult to identify potentially hereditary cases. For all these reasons, and principally because of the high associated malignant potential of resulting tumors, all the current algorithms used to guide genetic diagnoses include testing of *SDHB* in patients with PCC/PGL.

In a study of 64 pediatric PCC/PGL patients with *SDHB* germline mutations, most of the patients (78.13%) presented with extra-adrenal sympathetic tumors, and median size of the primary tumor was 5.7 cm. Metastases developed in 70% of patients at a median age of 16 years and were t diagnosed either in the first 2 years or in years 12–18 post-diagnosis. Around 19% of pediatric patients with *SDHB* mutation-related PCC/PGL presented with metastatic disease at the initial diagnosis, which warrants whole body studies to be performed at initial imaging evaluation. Thorough follow-up is crucial in the first 2 years post-diagnosis, and more frequent follow-ups are needed in years 10–20 post-diagnosis due to the increased risk of metastases. Most common site of metastases were bones, followed by the retroperitoneum and lungs. The estimated 5-, 10-, and 20-year survival rates were 100%, 97.14%, and 77.71%, respectively. Although this age group developed metastasis as early as 5 years from diagnosis, the overall 20-year prognosis and survival are good [6].

Carrying a mutation in *SDHB* has been associated with an increased risk of developing ccRCC, 4.7% at 60 years [62, 67], and so it is generally recommended not only that mutation carriers be screened for this disease but that mutation carriers with ccRCC be clinically worked up to rule out the existence of PGL.

Although the earliest reported age at diagnosis is 6 years, screening is recommended from age 5 years with initial work-up focusing on abdominal region [2]. If abdominal imaging is negative, evaluation of pelvic, chest, and head and neck

regions needs to follow. Abdominal MRI is recommended every 18 months with MRI of the neck, thorax, and abdomen and pelvis every 3 years. Currently, ^{68}Ga-DOTATATE PET/CT shows the highest per-lesion detection rate (93.5%) of primary and metastatic lesions compared to ^{18}F-FDG PET/CT and CT/ MRI scans. However, the use of ^{68}Ga-DOTATATE PET/CT seems to be limited to non-abdominal, especially bone, lesions probably due to reduced expression of SSTR2 in abdominal PCC/PGL [6].

Clinical Presentation Associated with Mutations in *SDHC*

Since relatively few mutations in *SDHC* (1q23.3) have been described worldwide, the associated clinical manifestations have not been clearly defined; nevertheless, it is known that mutation carriers tend to develop PGL (93% parasympathetic and 7% sympathetic) and infrequently adrenal PCC or GIST. Tumors are generally benign, although it has also identified metastatic extra-adrenal PGLs [68]. Seventeen percent of affected individuals have multiple PGLs, and 25% have a family history, suggesting that mutations have incomplete penetrance [23–25]. In fact, it has been shown that the estimated risk for *SDHC* non-probands carriers is 25% at 60 years of age [62]. While very little is known about the involvement of this gene in pediatric disease, the mean age at diagnosis of 43 years suggests that its genetic testing might not be a priority in cases with no family history of PGL [69].

Screening was recommended starting at 10 years of age for patients carrying a mutation in *SDHC* [64].

Clinical Presentation Associated with Mutations in *SDHA*

Based on the currently available information, *SDHA* (5p15.33) appears to account for approximately 3% of PCC/PGL [57]. These carriers had developed PCC, head and neck PGL, or thoracic and abdominal PGL (Table 5.2). One of the peculiarities of *SDHA* is that the mutations described to date have been reported to have low frequencies in unaffected population controls; this finding indicates that these mutations have incomplete penetrance and adds additional complexity to the genetic counseling offered to carriers. Nevertheless, *SDHA* should be considered in genetic testing for patients presenting with clinical evidence of familial PCC/PGL who test negative for the other known susceptibility genes. As previously mentioned, mutations in any of the SDH genes have the effect of suppressing the enzymatic activity of Complex II, and a key indicator that this has occurred is to detect negative immunostaining for SDHB. Furthermore, mutations in *SDHA* also give rise to negative immunostaining for SDHA [57]. This relatively easily implemented clinical screening tool should be incorporated into molecular diagnostic protocols to ensure that appropriate mutation testing is carried out in the most efficient and cost-effective manner. Screening was recommended starting at 10 years of age for patients carrying a mutation in *SDHA* [64].

Clinical Presentation Associated with Mutations in *SDHAF2*

The gene *SDHAF2* (11q12.2) is similar to *SDHD* in that it has an autosomal dominant mode of inheritance but is subject to maternal imprinting. To date, only head and neck PGLs have been reported in *SDHAF2* mutation carriers, most diagnosed at an early age and all with a family history of the disease ([54] and references contained therein). Available data suggest that mutations in *SDHAF2* do not explain a substantial portion of cases. Nevertheless, genetic testing of *SDHAF2* should be offered to patients with head and neck PGLs with negative tumor staining for SDHB and who test negative for mutations in *SDHD*, *SDHC*, and *SDHB*. Only two distinct *SDHAF2* mutations have been described in five independent families [54, 70–72]. While currently too few data are available to draw clear conclusions, none of the affected mutation carriers developed PGL before age 20 years, suggesting a priori that mutations are not relevant to the development of pediatric tumors.

FH: Clinical Presentation Associated with Mutations in *FH*

FH is the Krebs cycle enzyme involved in the reversible hydration/dehydration of fumarate to malate. It is known that germline mutations in *FH* (1q43) predispose to leiomyomas and ccRCC in an autosomal-dominant hereditary syndrome named hereditary leiomyomatosis and renal cell cancer (HLRCC) [73]. Loss-of-function mutations of *FH* lead to the accumulation of fumarate in the tumors which, like succinate, promotes the inhibition of the αKG-dependent dioxygenases [74]. In 2013, Letouze et al. [75] identified a germline mutation in *FH* by WES applied to blood and tumor DNA obtained from a 63-year-old female presenting with one PCC. The patient was selected to be sequenced because the tumor showed a methylome- and transcriptome-based profile very similar to that found in tumors carrying mutations in the SDH genes. The subsequent screening of almost 600 patients with PCC/PGLin whom no mutations in the major susceptibility genes had been found, revealed that five carried pathogenic germline *FH* mutations, providing further evidence of the involvement of this gene in the development of PCC/PGL [76]. Clinically, metastatic phenotype and multiple tumors were significantly more frequent in patients with *FH* mutations than those without such mutations. *FH* should thus be added to the list of PCC/PGL susceptibility genes and should be considered in mutation screening, to assess the risk of malignant disease.

5.8.2 *Non-syndromic PCC/PGL Associated with Mutations in* TMEM127

5.8.2.1 The TMEM127 Gene and its Protein

TMEM127 (2q11) was identified as a new PCC susceptibility gene in 2010, via an integrated analysis of results from studies using several genomic platforms, including linkage analysis, gene expression profiling, and mapping of chromosomal gains and losses [77]. Loss of heterozygosity (LOH) of the wild-type allele was observed in all available tumors from carriers of *TMEM127* mutations, suggesting that the gene acts as a classic tumor suppressor.

TMEM127 encodes a transmembrane protein with no known functional domains. Functional studies suggest that the protein (TMEM127) localizes to the plasma membrane and cytoplasm and is associated with a subpopulation of vesicular organelles, including the Golgi and lysosomes. TMEM127 is dynamically distributed at the subcellular level in response to nutrient signals [77]. It has also been demonstrated that TMEM127 modulates mTOR Complex 1 (mTORC1), which promotes cell growth and the translation of proteins and the phosphorylation of 4EBP1 and S6K. A detailed analysis of the global expression profile of *TMEM127*-mutated tumors grouped them with those associated with *RET* and *NF1* mutations.

5.8.2.2 Clinical Presentation Associated with Mutations in *TMEM127*

Few studies have been published to date based on patient series genetically tested for mutations in *TMEM127*. The most relevant of these reported the genetic findings in 990 patients with PCC or PGL who tested negative for mutations in *RET*, *VHL*, and *SDHD/B/C* [78]; 2% carried germline *TMEM127* mutations and presented with disease at a mean age of 43 years. Subsequent reports have described two mutation-carrying patients with PGL, one thoracic and abdominal and the other with multiple head and neck tumors. In addition, patients with renal carcinoma have been described, which would have an impact on surveillance and management of *TMEM127* mutation carriers [79, 80]. As for other susceptibility genes, the findings published to date suggest that mutations have incomplete penetrance, which would tend to mask the underlying hereditary disease and, in many cases, mean that patients may not meet the selection criteria for genetic testing. Given the mean age at disease onset for mutation carriers studied to date and the reported absence of bilateral disease and family history, genetic testing of *TMEM127* is not recommended in pediatric patients with PCC/PGL.

5.8.3 *Non-syndromic PCC/PGL Associated with Mutations in* MAX

5.8.3.1 The *MAX* Gene and its Protein

MAX (14q23.3) encodes a transcription factor that plays an important role in the regulation of cell proliferation, cell differentiation, and apoptosis, as part of the MYC/MAX/MXD1 axis. These proteins form dimers that bind to DNA; in fact, MYC forms a heterodimer with MAX to bind to specific DNA sequences called "E-boxes," which are located in MYC target genes, and this entire complex acts as a transcription activator. Both the lethal character demonstrated in *MAX*-knockout mice and the fact *MAX* is constitutively expressed in many cell types make it difficult to understand how MYC can carry out its function without the presence of MAX. However, the PC12 cell line, derived from PCC in rat, carries a homozygous *Max* mutation, which points to the existence of an additional unknown factor that is able to regulate the function of MYC (reviewed in [81]).

The identification of *MAX* as a PCC susceptibility gene was the result of a study of WES of three unrelated patients with PCC and a family history of the disease [81]. These patients had been selected because their tumors had a common transcription profile that differentiated them from tumors related to other known susceptibility genes [56]. LOH in the tumors of germline *MAX* mutation carriers, along with the absence of MAX protein shown by an immunohistochemical analysis, suggested that *MAX* acts as a tumor suppressor gene.

5.8.3.2 Clinical Presentation Associated with Mutations in MAX

Following the identification of pathogenic mutations in *MAX* in the three initial families, the genetic study was extended to 59 patients that had tested negative for the key known susceptibility genes. These 59 patients were chosen because they were diagnosed with PCC before age 30 years, had bilateral disease, or had a family history of the disease. *MAX* mutations were found in 8.5% of them; 67% of mutation carriers had bilateral disease, and 25% had developed metastases. One of the most striking findings was that the mutated allele had to have been inherited paternally in order for the carrier to develop the disease, as is the case for *SDHD* and *SDHAF2*, although the mechanism behind this remains unknown.

A subsequent study screened for mutations in *MAX* in a series of 1694 patients and 245 tumors in order to establish their prevalence and the associated clinical presentation. This study was made possible through the collaboration of 17 reference centers from around the world [82]. Pathogenic germline mutations were identified in 1.3% of patients; 21% of them had developed thoracic and abdominal PGL in addition to PCC, 37% had a family history of the disease, and 10% had metastases. The mean age of diagnosis for mutation carriers was 32 years, and 21% were diagnosed at or before age 18. These findings suggest that *MAX* should be included

along with *VHL* and *SDHB* in genetic testing protocols for pediatric cases [26]. This study also established that the frequency of somatic mutations is 1.65% and that the associated biochemical-secretor profile is characterized by elevated levels of normetanephrine and associated with normal or slightly increased levels of metanephrine [82]. Later, somatic *MAX* mutations have been found in many other cancers such as small cell lung cancer, GIST, multiple myeloma, and Wilms' tumors [83–86].

5.8.4 Non-syndromic PCC/PGL Associated with Mutations in Other Recently Identified Genes

During the last 5 years, several genes have been found to be involved in the hereditary predisposition to PCC/PGL, and so far, no mutations affecting these new genes have been found in pediatric cases. However, it is too soon to know which is their role in the pediatric presentation of PCC/PGL.

Although the first germline mutation found in one of the members of the Egl-9 family of hypoxia-inducible factors (EGLN) was reported in 2008 [87], recently two new variants have been reported in patients with PCC/PGL. Thus, a total of three germline mutations, two in *EGLN2* and one in *EGLN1* (also known as *PHD2* and *PHD1*, respectively), have been described in patients with PCC/PGL-polycythemia disorder [88]. Mutations in these genes cause substantial loss of protein stability of both PHD1 and PHD2, resulting in the upregulation of HIF-α target genes and therefore in the activation of hypoxic pathway.

MDH2 encodes malate dehydrogenase 2, which is essential for the reversible oxidation of malate to oxaloacetate in the TCA cycle. This tumor suppressor gene was first reported mutated, with an incomplete penetrance, in a single family with multiple malignant PGLs [89]. Later, the same mutation was found in another patient with malignant PCC, and additional pathological variants have been also reported accounting for <1% of the patients [90].

In 2018, germline mutations in the tumor suppressor gene *SLC25A11* were identified in seven unrelated patients, many of them with metastatic thoracic and abdominal PGLs. *SLC25A11*-mutated tumors showed a reduction of αKG levels with the pertinent accumulation of aspartate as a consequence of the malate-aspartate shuttle disruption. *SLC25A11* has been classified into the transcriptional Cluster 1A due to the SDHx-like molecular phenotype exhibited by the mutated tumors (i.e., pseudohypoxia and a CpG island methylator phenotype [CIMP] profile). *SLC25A11* gene mutations could account for 1% of all PCC/PGL [91]. Interestingly, a gain-of-function mutation in the *GOT2* gene, encoding the mitochondrial aspartate aminotransferase, was also reported in a PGL patient [92], further linking dysfunction of the malate-aspartate shuttle to PCC/PGL development.

Trio-based WES applied to the germline DNA of a selected patient strongly suspected of having hereditary PCC/PGL identified a single, de novo mutation in the

DNA methyltransferase 3A gene (DNMT3A) [93]. Genome-wide methylome analysis of *DNMT3A*-mutated tissues identified a characteristic CIMP profile as well as a significant hypermethylation of homeobox-containing genes, suggesting an activating role of the mutation. The extension of the study to a series of PCC/PGL patients and tumors revealed the presence of somatic sub-clonal mutations affecting the same residue in six additional tumors, all of them PGLs, and a second germline *DNMT3A* mutation (c.952C>T; p.Arg318Trp) in a patient with family history of PCC.

Finally, targeted sequencing of a panel of TCA cycle-related genes allowed the identification of germline variants affecting the dihydrolipoamide S-succinyltransferase (*DLST*) gene in seven unrelated patients [94]. A recurrent mutation (p.Gly374Glu) found in four unrelated patients with multiple PCCs/PGLs disrupted the TCA cycle triggering the accumulation of 2-hydroxyglutarate. In addition, p.Gly374Glu-*DLST* tumors exhibited LOH (by means of uniparental disomy), highly positive DLST immunostaining, as well as homogeneous expression and methylation profiles.

5.9 Sporadic PCC/PGL

5.9.1 PCC/PGL with Mutations in EPAS1

The HIF family of transcription factors (HIF-1α, HIF-2α [EPAS1], and HIF-3α) plays a key role in the regulation of hypoxia response to counteract the lack of oxygen in normal homeostasis. HIF-1α has been suggested to preferentially drive genes implicated in apoptosis and glycolysis, while HIF-2α is involved in cell proliferation and angiogenesis [95, 96]. A few years ago, a new and direct link between HIF proteins and PCC/PGL development has been found [27]; post-zygotic somatic mutations in *EPAS1* (2p21) were found in two unrelated patients with multiple PGLs, somatostatinomas, and polycythemia. The mutations were found in the residues located close to the prolyl hydroxylation site of the protein (proline 531) which was shown to disrupt the recognition of EPAS1 by the PHD family members, its hydroxylation, and the consequent degradation by VHL [97, 98]. Thus, mutations affecting the *EPAS1* gene stabilize the protein, causing the aforementioned pseudohypoxia, indicating that *EPAS1* behaves as an oncogene. A germline alteration affecting *EPAS1* was found in a patient with multiple PGLs and polycythemia. Although it has been demonstrated that the variant stabilizes the protein, its location outside the prolyl hydroxylation sites and the absence of segregation with the disease in the carrier's family make this result somewhat controversial [99].

5.9.2 *PCC/PGL with Mutations in* HRAS

The members of the RAS family of oncoproteins (e.g., *HRAS*, *NRAS*, and *KRAS*) are small GTP-binding proteins that affect multiple downstream pathways related to cell growth and homeostasis. They were first linked to cancer in 1982 [100], and nowadays it is known that together they represent around 30% of the total oncogenic activating mutations distributed across many different cancers [100, 101]. Mutations in *KRAS* appear in 21.6% of human cancers, *NRAS* is mutated in 8.0% of tumors, and *HRAS* mutations are found in 3.3% of cancers (www.sanger.ac.uk/genetics/CGP/cosmic/) [102]. A mutation affecting *HRAS* (11p15.5) was first described in one PCC by Yoshimoto et al. [103]. Crona et al. [104] applied WES to 58 PCCs and found that four harbored somatic mutations in the gene. The subsequent study of a large series of tumors determined that 10% of sporadic PCCs have mutations in *HRAS* and ruled out the involvement of *NRAS* and *KRAS* in the disease [105]. The presence of mutations in one of the isoforms of RAS is not a new issue in the development of endocrine tumors since they are present in around 10–20% of follicular cell-derived thyroid cancers and in 18% of *RET*-negative sporadic MTCs [106–108]. The pivotal role of RAS genes in the PIK3CA-AKT1-mTOR pathway explains why they group within the so-called transcriptional Cluster 2 [109].

5.9.3 *PCC/PGL with Mutations in* ATRX

The presence of somatic loss-of-function mutations in *ATRX* (alpha thalassemia/mental retardation syndrome X-linked) in PCCs/PGLs was first described in 2015 mostly coexisting with SDHx mutations (and therefore associated with Cluster 1A) [110]. However, there are also cases without any further driver mutation which are related to the transcriptional Cluster 3, which includes Wnt signaling-related tumors [109, 111]. Mutations in *ATRX* have been associated with alternative lengthening of telomeres and clinically aggressive behavior, and a recent study suggests that they are independent risk factors for metastatic PCC/PGL [112].

5.9.4 *PCC/PGL with Rearrangements Affecting* MAML3

The Cancer Genome Atlas (TCGA) project revealed PCCs/PGLs carrying somatic gene fusions affecting the *MAML3* (mastermind-like transcriptional coactivator 3) transcription factor gene, with increased transcription levels of a chimeric MAML3 [109]. One of the fusions observed in PCCs/PGLs, UBTF-MAML3, leads to the activation of the Wnt target expression and Hedgehog signaling pathway, something already detected in neuroblastoma, a tumor with a similar developmental origin to

PCCs/PGLs. Another important finding of this study is that the presence of MAML mutations in PCCs correlates with poor clinical outcome.

5.9.5 Other Somatic Mutations Observed in PCC/PGL

Somatic mutations in the *IDH1* gene, frequently found in central nervous system tumors [113], have been also identified in PCCs/PGLs leading to a neomorphic production of D-2HG that finally causes the characteristic CIMP profile. However, they are low-frequent events in PCC/PGL (<1%) [92, 109, 114]. Very recently, a single HN-PGL carrying a somatic mutation in IDH2 has also been reported [115].

In the TCGA study, somatic loss-of-function mutations in *CSDE1* (cold shock domain containing E1) were also reported. *CSDE1* encodes an RNA-binding protein not hitherto associated with cancers [109].

Postzygotic mosaic mutations in *H3F3A* (H3 histone family member 3A) cause PCC/PGL together with giant cell tumor of the bone and lead to the upregulation of MYCN [116]. *H3F3A*-mutated PCCs/PGLs have been proposed to be part of Cluster 2, although due to their function they may fit better into Cluster 1. Other chromatin-remodeling genes found mutated in PCCs/PGLs are *EZH2*, *HIST1H1T*, *HIST4H4*, *JMJD1C*, *KDM2B*, *KMT2B*, or *SETD282*.

5.9.5.1 Treatment

Surgery is the treatment of choice for both PCC and sympathetic PGL. For PCC, laparoscopic intervention has a lower associated morbidity and mortality than open procedures. Laparoscopic option is not contraindicated for large, multiple, bilateral, malignant, or recurrent tumors, and the final decision regarding the surgical approach usually depends on the experience of the surgeon. An alternative for bilateral complete adrenalectomy in hereditary PCC is bilateral partial adrenalectomy (also known as subtotal, function-preserving adrenalectomy or adrenal sparing surgery). This alternative should be raised with the patient, considering their advantages (avoid the adrenal insufficiency and other morbidities associated with long-term corticotherapy) and disadvantages (greater risk of recurrence and remaining possibility of adrenal insufficiency). Their use in cases of sporadic unilateral PCC in patients with previous damage of the contralateral adrenal gland remains controversial [3, 13].

Patients with head and neck PGL are generally differentiated into (1) those with asymptomatic small-medium-sized tumors for which ongoing observation is usually indicated and (2) those with large, symptomatic, or fast-growing tumors, for which both surgery and radiotherapy/radiosurgery are viable options. The therapeutic decision depends on the center, the patient, and the possible side effects predicted [117, 118].

The therapeutic options for metastatic cases are limited and rarely curative. Surgery has not shown to improve survival, but it can reduce the size of the mass that produces catecholamines or that produces local compression and can be used as an adjuvant treatment to radiotherapy or chemotherapy. In cases where surgical resection is not viable, who are positive on 123I-MIBG scintigraphy and have slowly growing metastatic lesions, radiotherapy with *^{131}I is recommended but with the goal of maintaining disease stability and less for regression or disease cure.* A new preparation of ^{131}I-MIBG, produced on the Ultratrace® platform, may increase tumor uptake and treatment efficacy, but the spectrum of side effects on the Ultratrace® platform has yet to be presented.

If the ^{123}I-MIBG scintigraphy is negative and/or the tumor is fast-growing, chemotherapy (cyclophosphamide, vincristine, dacarbazine CVD) alone or, in combination with radiotherapy, is an option in malignant disease in both pediatric and adult cases [119].

Our improved understanding of the molecular biology of these tumors has helped to broaden the therapeutic options. Anecdotal reports have suggested several other treatment approaches, a few of which deserve further evaluation:

(a) Pro-apoptotic: somatostatin analogues, histone deacetylase (HDAC) inhibitors, eicosapentaenoic acid, triptolide/capsaicin, gamitrinib, and camptothecin.
(b) Anti-proliferative: everolimus (mTOR1 inhibitor), AEZS-131 (ERK inhibitor), AZD-8055 (mTOR1 and mTOR2 inhibitor), sunitinib and other tyrosine kinase inhibitors (pazopanib, axitinib, and cabozantinib), LB1 (inhibitor of serine/threonine protein phosphatase 2A) combined with temozolomide, and inhibitors of carboxypeptidase E [119].
(c) DNA methyl transferase inhibitors: guadecitabine.
(d) Checkpoint inhibitors.
(e) Topoisomerase or PARP inhibitors.
(f) Glutaminase inhibitors [12].

Other treatment options include radiotherapy to alleviate pain or symptoms due to local compression (especially for bone metastases) and local treatment with cryoablation, radiofrequency ablation, radionuclides, and/or embolization [9, 13]. Because of the lack of curative treatments, most pediatric patients are treated only if they present with reduced quality of life [16].

5.9.5.2 Perioperative Clinical Management

For all patients with elevated norepinephrine or metanephrine, the present-day recommendation is to offer preoperative pharmaceutical "blockade" regardless of symptoms for at least 7–10 days before proceeding with the surgery.

Appropriate clinical management of patients, including those with non-functional disease, is essential to prevent intraoperative hypertensive crises and minimize the adverse effects of the anesthesia and tumor manipulation (Table 5.3) [13, 120]. Randomized controlled trials have not been conducted, and therefore no consensus

Table 5.3 Preoperative management

Condition	Treatment	Characteristics
Hypertension	α-Adrenergic blockers	
	– Phenoxybenzamine 0.2 mg/kg/day (max. 10 mg/dose) Increase by 0.2 mg/kg/day every 4 days to goal 0.4–1.2 mg/kg/day divided every 6–8 h (max. 2–4 mg/kg/day)	– α1- and α2-adrenergic blocker that is irreversible, non-selective, non-competitive, and long-acting – Side effects: Nasal stuffiness, fatigue, dizziness, reflex tachycardia, retrograde ejaculation, orthostasis and hypotension (up to 36 hours following surgery) – Contraindicated in cardiopulmonary disease
	– Prazosin, terazosin, doxazosin. 1–2 mg/day Increased to 4–16 mg, daily or divided two times daily	– α1-adrenergic blocker that is reversible, selective, competitive, and short-acting – Treatment of choice for cases requiring long-term treatments – Higher risk of intraoperative complications: The binding to the receptor may be lost if an abundant amount of catecholamines is released – Lower risk of postoperative hypotension, tachycardia, and side effects
	Calcium channel blockers	
	– Dihydropyridines: Slow-releasing nifedipine and nicardipine – Non-dihydropyridines: Diltiazem	– Used to supplement α-adrenergic blockers if blood pressure remains uncontrolled or as a second-line treatment if side effects are not tolerated
	Competitive inhibitor of *tyrosine hydroxylase*	
	Metyrosine 20 mg/kg/day, divided every 6 h or 125 mg daily Increase up to 60 mg/kg/day divided every 6 h or increase by 125 mg every 4–5 days to max. 2.5 g/day	– Normally used only when other treatments have been ineffective or poorly tolerated or in cases of metastatic or inoperable disease, cardiopulmonary disease or where substantial tumor manipulation is foreseen – Side effects: Sedation, diarrhea, extrapyramidal signs, nightmares, depression, urolithiasis, and galactorrhea
Hypertensive crisis	– Short-acting α-adrenergic blockers such as intravenous phentolamine. Not recommended where there is risk of *cardiogenic shock* – Vasodilators such as nitroprusside, nicardipine, fenoldopam, or nitroglycerin – Magnesium sulfate, typically as a second-line treatment	

(continued)

Table 5.3 (continued)

Condition	Treatment	Characteristics
Angina, arrhythmia, or reflex tachycardia	Cardioselective β-adrenergic blockers: Propranolol 1–2 mg/kg/day, divided 2–4 times daily 4 mg/kg/day, up to 640 mg/day, divided 2–4 times daily Atenolol 0.5–1 mg/kg/day, daily or divided two times daily 2 mg/kg/day, up to 100 mg/day, daily or divided two times daily Labetalol 1–3 mg/kg/day, divided 2–3 times daily 10–12 mg/kg/day, up to 1200 mg/day, divided 2–3 times daily Metoprolol or bisoprolol	– If an adequate α-adrenergic blocker has not been previously initiated, β-adrenergic blockers can cause hypertensive crisis and, in the case of underlying cardiomyopathy, acute pulmonary edema Can be started at least 3 days prior to surgery. Common sides effects: Dizziness, fatigue, and asthma exacerbation
	Esmolol or lidocaine	– In the event of intraoperative tachycardia
Intravascular volumen expansion	Abundant oral intake of fluids. Intravenous infusion of 2–3 liters of saline the day before surgery. Diet high in salt content (6–10 g) and in some cases prescription of sodium chloride tablets. Caution should be taken with children, patients with heart or kidney disease and patients with increased risk of pulmonary edema.	

protocol exists for the medical management of these tumors in adults or children; and the procedures followed in most cases depend on the experience of the institution involved. The goal is blood pressure reduction of <50 percentile for age and height. Dilated cardiomyopathy can develop from chronic catecholamine-induced hypertension, making an echocardiography valuable preoperatively.

The possibility of particular postoperative complications, apart from the common ones such as hemorrhage, hematoma, and infection, should also be taken into account (Table 5.4). In head and neck PGLs, due to their proximity to vascular and nerve structures, the resection can give rise to specific complications.

5.9.5.3 Clinical Follow-Up

In order to ensure that the resection has been complete, biochemical testing is recommended 2–6 weeks after surgery, depending on patient recovery. If persistent elevation is observed, it is important to determine whether this is due to residual tumor, occult metastases, or the presence of additional primary tumors.

Smaller pediatric and adult case series recommend follow-up at 6 weeks and between 6 months and 1 year following initial surgery and then annually. All patients with genetic mutations should be followed throughout their lifetime given the risk of recurrence and malignancy. A multidisciplinary management including

Table 5.4 Postoperative complications [5, 7, 10, 11]

Complication	Causes
Hypotension	The cause may be multifactorial: – Loss of peripheral vasoconstriction – Persisting effect of the drugs used in the preoperative work-up, particularly if phenoxybenzamine or metyrosine was taken – Blood volume depletion – Adrenocortical insufficiency
Hypertension or *blood pressure liability*	It is important to differentiate whether the occurrence or persistence of hypertension is due to: – Postoperative pain – Volume overload – Autonomic instability – Incomplete tumor resection – Existence of an undetected tumor – *Coexisting essential hypertension* – Accidental ligation of a renal artery or renal failure
Hypoglycemia	– Loss of the suppressive effect of catecholamines on the secretion of insulin – Adrenocortical insufficiency

endocrinologists, genetic counselors, radiologists, oncologists, and surgeons for the optimal follow-up is important.

All patients with PCC/PGL are at risk for tumor recurrence even after complete resection without residual disease, and the current WHO classification stresses that all PCC/PGLs have a metastatic potential [19].

Nevertheless, no universal consensus exists with regard to the biochemical or imaging tests to be used or the frequency with which they should be applied [2, 9]. Biochemical tests should be carried out at least annually, with a focus on the biochemical phenotype particular to the known mutation. Imaging tests are generally reserved for those cases with positive results in biochemical analyses and to monitor the development of non- or low-secreting tumors, in those with a higher risk especially TCA cycle-related PCC/PGLs. Periodic image testing is recommended for carriers of mutations in the SDH genes because of the possibility of developing non-functional PGLs; MRI is preferable because it is more sensitive in detecting extra-adrenal tumors and minimize radiation exposure.

5.10 Conclusions

Faced with the complex genetic scenario described in this chapter, in order to offer an appropriate and efficient genetic test to a patient, it is essential to collect information related to age at diagnosis, tumor location, bilaterality, multiplicity, family history of disease, and the development of metastases, as well as the biochemical and immunohistochemical characteristics of the tumor. Also necessary for a comprehensive molecular diagnosis is tumor DNA, since 30–40% of patients will have

somatic mutations mainly in *NF1*, *HRAS*, *VHL*, *or RET*. The detection of a germline or somatic mutation in one of the genes related to the development of these tumors has clear implications for genetic counseling and the clinical follow-up of the patient.

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Chapter 6
Wilms Tumor

Joyce T. Turner, Leslie A. Doros, and Jeffrey S. Dome

Abstract Wilms tumor (WT) is the most common renal tumor in pediatrics, representing 6.3% of all childhood cancers. It is a developmental neoplasm that arises from embryonic kidney precursor cells. Most WTs are sporadic, but about 10–15% of patients have clinical features that suggest the presence of a constitutional predisposing mutation. Such features include bilateral disease, family history of WT, and congenital anomalies, which can occur in isolation or as part of a defined syndrome. Our understanding of the molecular biology and genetics of WT originated with the discovery of the *WT1* gene in the early 1990s. It has since become apparent that WT is a complex genetically heterogeneous tumor in which multiple genetic and epigenetic alterations participate in tumorigenesis. To date, constitutional mutations in more than 20 different genes have been identified in individuals with WT. Research to further elucidate the genetics of WT is an active area of investigation.

Keywords Wilms tumor · WT1 · Nephrogenic rest · Imprinting · Congenital anomalies · Wilms-related syndromes

6.1 Introduction

Wilms tumor (WT) is the most common renal malignancy in pediatrics and the fifth most common cancer in children under 15 years of age. Approximately 500 new cases are diagnosed annually in the United States representing about 6.3% of childhood cancers [1]. Approximately 5–10% of patients have bilateral disease; for patients with unilateral disease, the median age of diagnosis is 44 months, and for those with bilateral disease, it is 31 months [2].

J. T. Turner
Division of Genetics, Children's National Hospital,
Washington, DC, USA
e-mail: JDome@childrensnational.org

L. A. Doros · J. S. Dome (✉)
Division of Oncology, Children's National Hospital,
Washington, DC, USA

D. Malkin (ed.), *The Hereditary Basis of Childhood Cancer*,
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Symptoms at presentation most commonly include a painless abdominal mass found by the parent or physician on routine examination. Elevations in blood pressure, hematuria, and abdominal pain can each occur in about 25% of the cases [3]. Multimodality therapy is most often needed and includes nephrectomy, chemotherapy, and radiation therapy, depending on the tumor stage at presentation. Long-term survival exceeds 90% for localized disease and is greater than 80% for metastatic disease [4]. Approximately 10% of tumors exhibit anaplastic histology, which confers a less favorable prognosis [5, 6].

WT has been a model for genetic studies of cancer development since the early 1970s. WT results from malignant transformation of renal stem cells that retain embryonic differentiation potential. It is now known that subsets of WT exhibit distinct gene expression profiles based on mutation patterns and the stage of embryonal cell differentiation at which the mutation occurred [7]. Although WT was one of the original tumors upon which Knudson based his "two-hit" model of tumorigenesis, the development of WT is complex and is likely to involve multiple genetic alterations. Numerous genes have been implicated in the pathogenesis of WT, some associated with constitutional mutations only, some associated with somatic mutations only, and others associated with both constitutional and somatic mutations [8, 9].

The most common somatic alterations that have been observed in WT converge on several pathways involved in renal development: transcriptional regulation (*WT1*, *MYCN*, *SIX1*, *SIX2*, and *MLLT1*, collectively found in ~20–25% of WT), microRNA (miRNA) processing (*DGCR8*, *DROSHA*, *DICER1*, and *XPO5*, collectively found in ~15–20% of WT), and WNT signaling (*CTNNB1* and *AMER1*, collectively found in ~30–45% of WT) [8, 10, 11]. While uncommon in WT overall, somatic *TP53* mutations are detected in 50–75% of anaplastic histology WT [8, 12, 13]. Additionally, approximately 70% of WT show evidence of increased IGF2 expression, which may arise via genetic or epigenetic changes [7, 10]. IGF2 is thought to contribute to but not be sufficient for Wilms tumorigenesis.

This chapter focuses on the constitutional genetic alterations that predispose to WT. Approximately 10% of WT are associated with constitutional mutations or epigenetic alterations involving more than 20 genes or loci (Table 6.1). *WT1*, *TRIM28*, *REST*, and 11p15 epimutations/uniparental disomy each account for approximately 2% of cases of WT with the remaining genes are very rare and collectively account for about 2% of cases of WT [9]. The constitutional mutations may occur with or without syndromic features.

6.2 Syndromic Wilms Tumor

6.2.1 WT1-Related Syndromes

A variety of germline *WT1* mutations have been described, including missense mutations, deletions, insertions, and splice-site events. Together they have come to be described as a spectrum of disorders [14, 15]. These different types of mutations

Table 6.1 Constitutional mutations seen in patients with Wilms tumor

Gene	Constitutional mutations in unselected patients (%)	Constitutional mutation in families with a history of WT (%)
WT1	2	6
TRIM28	2	8
IGF2	2	–
REST	2	8
CTR9	Rare	5
H19 hypermethylation	Rare	3
CDC73	Rare	1.6
Biallelic *BRCA2*	Rare	1.6
Biallelic *NYNRIN*	Rare	1.6
All others: *ASXL*, *DICER1*, *FBXW7*, *KDM3B*, *TP53*, *BLM*, *BUB1B*, *DIS3L2*, *PALB2*, *TRIM37*, *TRIP13*, *GPC3*, *PIK3CA*	Collectively <2%	Rare

Adapted from Mahamdallie et al. (2019) [9]

lead to distinct phenotypic features, including deletions, which cause WAGR syndrome; missense mutations, which cause Denys-Drash syndrome; and splice-site mutations, which result in Frasier syndrome. The risk of developing WT depends on the type of genetic alteration and varies with each syndrome, as described below. Somatic *WT1* mutations that include stop and frameshift mutations occur in 10–20% of sporadic WT. Individuals with unilateral WT and no congenital anomalies are less likely to have a constitutional *WT1* mutations (<5%), and patients with constitutional mutation are more likely to have bilateral or multifocal disease [16].

6.2.1.1 WAGR Syndrome

Cytogenetic studies in the 1960s and 1970s revealed that WAGR syndrome, a constellation of WT, *a*niridia, *g*enitourinary abnormalities, and a *r*ange of developmental delays, is associated with constitutional deletions of chromosome 11p13. In 1990, the *WT1* gene was identified as the gene responsible for the genitourinary anomalies and WT predisposition [17, 18]. Deletions of this locus also involve the *PAX6* gene responsible for aniridia. The severity of this condition varies depending on the size of the deletion. In addition to Wilms tumor, aniridia, genitourinary malformations, and developmental disorders, affected individuals may experience focal segmental glomerular sclerosis, obesity, thought to be due to the deletion of the *BDNF* gene, and other possible medical issues [19–22]. *WT1* encodes a zinc-finger transcription factor that plays a critical role in regulating other genes responsible for the development of the genitourinary system [16, 23]. Individuals with WAGR syndrome have a 30–60% risk of developing WT, yet WAGR syndrome is observed in only about 0.4–0.75% of children with WT. The incidence of bilateral disease among patients with WAGR syndrome is approximately 14–20% [20, 24, 25].

6.2.1.2 Denys-Drash Syndrome

Denys-Drash syndrome (DDS) is a rare autosomal dominantly inherited disorder with approximately 150 cases reported worldwide. It is characterized by the triad of incomplete male genital development, progressive glomerulopathy (diffuse mesangial sclerosis), and WT. While males can have normal genitalia, they typically have gonadal dysgenesis or ambiguous genitalia. The testes can be undescended, but they can also have complete sex reversal. Females typically exhibit normal genitalia and develop early onset/infantile nephropathy. Unlike the cytogenetic deletions found in WAGR, individuals with DDS typically harbor missense mutations in *WT1* in exons 8 or 9, which affect the zinc-finger domains implicated in DNA binding [16, 23, 26, 27]. The risk of developing WT is estimated to be over 70% [28].

6.2.1.3 Frasier Syndrome

Frasier syndrome is an autosomal dominantly inherited disorder associated with nephropathy, gonadal dysgenesis, and gonadoblastoma. Genetic males typically have incomplete sexual development or complete sex reversal and appear as phenotypic females. They usually present with nephropathy and/or gonadoblastoma. Genetic females usually have normal genitalia and present with nephropathy. WT is infrequently seen in association with Frasier syndrome. *WT1* mutations have been found in patients with Frasier syndrome and occur as germline point mutations in the intron 9 donor splice site [23, 29, 30]. The *WT1* mutations that cause Frasier syndrome lead to an altered ratio of WT1 protein isoforms with impaired ability to control gene activity and regulate the development of the kidneys and reproductive organs [29, 30].

6.2.2 Overgrowth Syndromes

Evidence of increased susceptibility to WT has been demonstrated in several childhood overgrowth syndromes. The most completely characterized overgrowth syndrome is Beckwith-Wiedemann syndrome (BWS), but other overgrowth syndromes include the Simpson-Golabi-Behmel, Perlman, Sotos, and *PIK3CA*-related overgrowth syndrome (PROS) [23, 31–35]. Here we focus on the association of overgrowth syndromes with WT.

6.2.2.1 Beckwith-Wiedemann Syndrome

BWS is now described as a spectrum of disorders [31] and is the most common epigenetic overgrowth syndrome associated with Wilms tumor and other embryonic tumors. It affects ~1/10,340 individuals [32, 36] and is composed of characteristic

clinical features including macroglossia, lateralized overgrowth (also called hemihypertrophy or hemihyperplasia), exomphalos/omphalocele, WT (multifocal, bilateral, or nephroblastomatosis), and hyperinsulinemia. Suggestive features of BWS include polyhydramnios, placentomegaly, macrosomia, large for gestational age (birth weight > 2 SD), neonatal hypoglycemia, facial nevus simplex, ear creases/pits, organomegaly (nephromegaly, hepatomegaly), umbilical hernia, diastasis recti, and various other embryonal cancers [31, 32, 37, 38]. The phenotypic subtypes, a proposed scoring system for diagnosing BWS, and recommendations for genetic testing were recently described [31].

Whereas cancer risk has historically been quoted at ~8%, more recent studies suggest a higher tumor risk of approximately 14.5%. This difference is attributed to mosaic BWS identified through tissue analysis, which has improved diagnostic yield from about 70% to over 80% [31, 32]. Among those developing an embryonal tumor, the most common type of tumor is WT, accounting for ~52% of tumors. Other tumors include hepatoblastoma (~14%), neuroblastoma (10%), rhabdomyosarcoma (5%), adrenocortical carcinoma (3%), and pheochromocytoma (<1%). The highest risk for development of these tumors is prior to 2 years of age, and this risk decreases around 8 years of age [31, 39]. There does not appear to be an increased risk for tumor development in adulthood associated with BWS.

BWS is caused by changes occurring in both growth-promoting and growth-suppressing genes. Family linkage studies conducted in the 1980s identified chromosome 11p15 as the locus responsible for BWS [40–42]. This locus contains several imprinted genes in which only one parental allele is normally expressed (Fig. 6.1). The genes are clustered into two domains or imprinting centers,

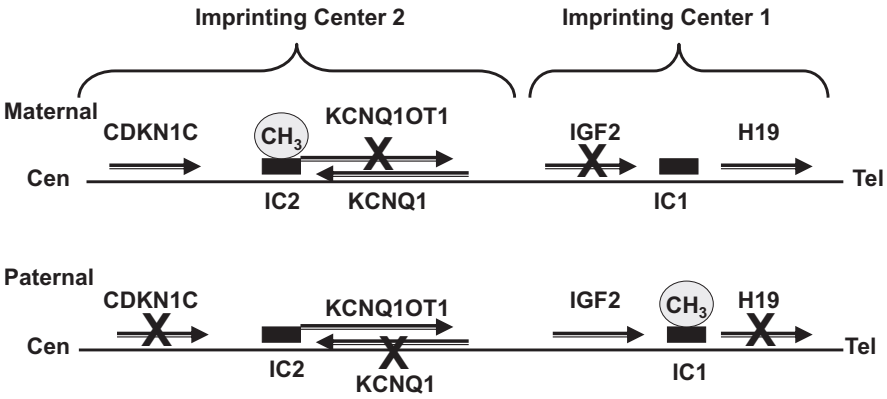

Fig. 6.1 The Beckwith-Wiedemann syndrome locus. The chromosome 11p15.5 locus contains several imprinted genes clustered in two imprinting centers, IC1 (telomeric (tel) and IC2 (centromeric (cen)). Each domain has a differentially methylated region that controls expression of surrounding genes. In normal individuals, the differentially methylated region of IC1 is methylated in the paternal allele, resulting in expression of *IGF2* and silencing of *H19*. In normal individuals, the differentially methylated region of IC2 is methylated in the maternal allele, resulting in expression of *CDKN1C* and *KCNQ1* and silencing of *KCNQ1OT*

commonly referred to as imprinting center 1 (IC1), previously described as differentially methylated region 1 (DMR1), and imprinting center 2 (IC2), also known as differentially methylated region 2 (DMR2) [40, 41, 43–47]. IC1 contains the insulin-like growth factor 2 (*IGF2*) and *H19* genes. *IGF2* encodes a growth factor and *H19* encodes an untranslated RNA of unclear significance. The paternal allele of IC1 is normally methylated, resulting in expression of *IGF2* and silencing of *H19*. IC2 contains several genes including *KCNQ1*, *KCNQ1OT1*, and the tumor suppressor gene *CDKN1C* (p57/K1P2). IC2 is normally methylated on the maternal allele, resulting in expression of *KCNQ1* and *CDKN1C* and repression of *KCNQ1OT1*. BWS may arise from various genetic and epigenetic changes at the 11p15 locus, each of which is associated with distinct phenotypes and cancer risk, as follows:

1. Loss of methylation (hypomethylation) of IC2 on the maternally derived chromosome (~35–50% of BWS cases) is associated with typical BWS facial features such as macroglossia, ear creases/pits, and facial nevus. Epigenetic defects of IC2 are also more frequently seen with prematurity, abdominal wall defects (omphalocele, umbilical hernia, and diastasis recti), and undescended testes. Individuals with this epigenetic finding have a low risk of WT (<1%) and other cancers (2.6%–4.4%) [32, 39]. Subfertility with or without the use of assisted reproductive technologies is associated with this subset of BWS.
2. Paternal uniparental disomy (pUPD11) (~18.5%–23% of BWS cases), in which the paternal allele recombines and replaces the maternal allele, affects both IC1 and IC2. This subtype has a high association with babies who are large for gestational age and who have lateralized overgrowth, hyperinsulinism, hypoglycemia, and a risk of developmental delay. The risk for WT in this group is approximately 8% [31].
3. Hypermethylation of IC1 (~5–9% of BWS cases) is frequently associated with babies who are large for gestational age and who have diastasis recti, as well as organomegaly (hepatomegaly, splenomegaly, and nephromegaly). Undescended testes are also common to this subgroup. Tumor incidence is greatest for those with IC1-associated BWS, with approximately 52% developing a tumor. This subgroup is associated with the highest chance for bilateral/multifocal WT or nephroblastomatosis (32%) and other tumors (19.4%) [31, 32].
4. Mutations of the maternal *CDKN1C* gene (2–5% of BWS cases, but 40% of familial BWS cases) tend to be affected by omphalocele and preterm birth. Changes in the *CDKN1C* gene confer a low risk of WT, but neuroblastoma tends to be more common among those with a change in this gene [31, 32, 41, 43, 48].
5. Various other genetic and epigenetic changes including genome-wide paternal UPD (GWUPD11), duplications, deletions, inversions, and translocations of 11p15 account for a small percentage of BWS cases (~3–6%) [31, 32].
6. Recommended molecular testing for BWS includes methylation testing, copy number variant testing, *CDKN1C* mutation analysis, SNP array to distinguish between pUPD and GWpUPD, and tissue analysis. While the International Consensus Group recommends different screening for BWS-associated tumors

based on the underlying epigenetic/molecular cause for BWS [31], other groups do not yet recommend epigenetic/molecular subtype-defined screening [32] (Table 6.2).

6.2.2.2 Simpson-Golabi-Behmel Syndrome

Simpson-Golabi-Behmel syndrome (SGBS) is an X-linked recessive condition. It can be caused by mutations in glypican-3 (*GPC3*) at Xp26, intragenic or whole-gene deletion of *GPC3*, which can include a portion or all of *GPC4*, or duplication of *GPC4*. Most cases arise from mutations in *GPC3* [49–51]. As *GPC3* is located on the X chromosome, female carriers are usually asymptomatic or have mild features due to skewed X inactivation.

GPC3 and *GPC4* encode cell surface heparan sulfate proteoglycans that interacts with the WNT signaling pathway and affects several growth factors [52]. *GPC3* is composed of eight exons. *GPC4* is positioned 3′ to *GPC3* and consists of nine exons [53].

SGBS is characterized by pre- and postnatal macrosomia, macrocephaly, macroglossia, macrostomia, coarse facial features, ear abnormalities (preauricular tags, creases, helical dimple, and hearing loss), skeletal defects (vertebral fusions, scoliosis, rib abnormalities, congenital hip dislocation, large hands with or without post axial polydactyly), cardiac abnormalities (septal defects, pulmonic stenosis, aortic coarctation, transposition of the great vessels, patent ductus arteriosus, and patent foramen ovale), mild to moderate intellectual disability, and genitourinary defects (nephromegaly, multicystic kidneys, hydronephrosis, hydroureter/duplicated ureters, bifid scrotum, cryptorchidism, hydrocele, and inguinal hernia). Additional features that can be seen included cleft lip and palate, supernumerary nipples, diastasis recti/umbilical hernia, heart defects, diaphragmatic hernia, and gastrointestinal anomalies (pyloric ring, Meckel's diverticulum, intestinal malrotation, hepatosplenomegaly, pancreatic hyperplasia, choledochal cysts, pancreatic duct duplication, and polysplenia). Affected individuals are at increased risk (5–10%) for embryonal tumors, including WT, hepatoblastoma, adrenal neuroblastoma, gonadoblastoma, hepatocellular carcinoma, and medulloblastoma [54, 55]. Approximately 9% of patients with SGBS due to *GPC3* mutations have developed WT [49, 50, 55].

6.2.2.3 Perlman Syndrome

Perlman syndrome (PS) is a rare autosomal recessively inherited disorder. The locus for PS has been mapped to chromosome 2q37.1, and germline-inactivating mutations have been identified in the *DIS3L2* gene, which encodes a protein involved in microRNA processing [56]. PS is characterized by congenital overgrowth nephromegaly with renal dysplasia, polyhydramnios, inverted V-shaped upper lip, prominent forehead, deep-set eyes, broad, flat nasal bridge, low-set ears, and developmental delay [57, 58]. PS has a high rate of neonatal mortality, but among individuals who

Table 6.2 Syndromes and conditions with Wilms tumor (WT) surveillance recommendations

Phenotype	Associated genetic alterations	Estimated WT risk	Duration of surveillance	Comments
WT1-related disorders	*WT1* deletion (WAGR syndrome)	50%	Abdominal US every 3 months until age 5 years	Some advocate extending the surveillance period with annual imaging if the kidneys have evidence of nephrogenic rests (WT precursors)
	WT1 missense mutations (Denys-Drash syndrome)	>70%		
	WT1 intron 9 splice site (Frasier syndrome)	Rare		
	WT1 missense/ nonsense mutations (isolated WT)	5%		
Beckwith-Wiedemann syndrome	11p15 uniparental disomy (pUPD11)	8%	Abdominal US every 3 months until age 7–8 years	Surveillance indicated for those with 11p15 pUPD11, IC1 hypermethylation, *CDKN1C* mutation, and BWS without identifiable genetic alterations; opinions are mixed whether surveillance is indicated for those with loss of methylation of IC2
	Hypermethylation of 11p15 imprinting center 1 (IC1)	24%		
	Loss of methylation of 11p15 imprinting center 2 (IC2)	<1%		
	CDKN1C mutation	1–2%		
	No molecular findings	4–5%		
Simpson-Golabi-Behmel syndrome	*GPC3*	5–10%	Abdominal US every 3 months until age 7–8 years	Screening also involves AFP levels for hepatoblastoma Males should undergo surveillance; female carriers are asymptomatic or have mild features of SGBS. WT among female carriers has not been reported
Perlman	*DIS3L2*	>60%	Abdominal US every 3 months until age 7–8 years	
PIK3C-*A*-related overgrowth syndromes	*PIK3CA* (mosaicism)	TBD	Consider abdominal US every 3 months until 8 years of age	
Mosaic variegated aneuploidy	*BUB1B* *TRIP13*	>25%	Abdominal US every 3 months until 5–7 years of age	

(continued)

Table 6.2 (continued)

Phenotype	Associated genetic alterations	Estimated WT risk	Duration of surveillance	Comments
Bohring-Opitz syndrome	*ASXL1*	7%	Abdominal US every 3–4 months until 8 years	
Fanconi anemia	Biallelic *BRCA2* mutation (Fanconi anemia D1) Biallelic *PALB2* mutation (Fanconi anemia N)	>20% 40%	Abdominal US every 3 months until age 7–8 years	Screening also involves brain imaging
DICER1 syndrome	*DICER1*	Infrequent	Abdominal US every 6 months until 8 years of age and then annually until age 12	Screening for WT in DICER1 is part of a larger screening protocol; abdominal US also used to look for cystic nephroma and renal sarcoma
Li-Fraumeni syndrome	*TP53*	Infrequent		Screening protocol for abdominal masses includes whole-body MRI once a year throughout one's life and abdominal US every 3 months until 18 years and then twice annually
Familial WT	*TRIM28* *FBXW7* *REST* *CTR9* *NYNRIN* *KDM3B* Various genes unidentifiable and others without identifiable loci	TBD	Abdominal US every 3 months until age 7–8 years	Surveillance should be offered to at-risk children who have more than one family member with WT or children of patients with bilateral WT

survive beyond the neonatal period, there is a 64% incidence of WT [58]. The tumor is diagnosed at an earlier age in these individuals compared with sporadic cases (usually less than 2 years of age), and there is a high frequency of bilateral tumors (55%) [57, 58]. Histological examination of the kidneys in children with PS frequently demonstrates nephroblastomatosis, which is a precursor lesion for WT [57].

6.2.2.4 Sotos Syndrome

Sotos syndrome is an autosomal dominant disorder. About 80–90% of individuals with Sotos syndrome type 1 have a demonstrable mutation or deletion of the nuclear receptor SET domain-containing protein 1 (*NSD1*) gene on chromosome 5q35. *NSD1* encodes a protein that belongs to a family of nuclear receptors that bind to DNA response elements for ligands such as steroid and thyroid hormones and retinoids [59]. Sotos syndrome type 2 is caused by a mutation in the *NFIX* gene on chromosome 19p13, and type 3 is caused by a mutation in the *APC2* gene also located on chromosome 19p13. To date, types 2 and 3 are not known to be associated with tumor predisposition. The diagnosis of Sotos syndrome is established by a combination of clinical findings and molecular genetic testing.

Sotos syndrome is an overgrowth condition with cardinal facial features including prominent forehead with receding hairline; down-slanting palpebral fissures; long narrow face with a long, pointed chin; and a large head circumference (>2 SD). It is also associated with mild to severe intellectual dysfunction and behavioral problems. Brain anomalies, seizures, cardiac anomalies, joint laxity, renal abnormalities, and scoliosis can be present as well [60, 61]. There is a 2–3% risk of developing a tumor including one of the following: WT, sacrococcygeal teratoma, neuroblastoma, ganglioma, acute lymphoblastic leukemia, small cell lung cancer, and astrocytoma [60, 62, 63].

6.2.2.5 PIK3CA-Related Overgrowth Spectrum (PROS)

PIK3CA-related overgrowth (PROS) is a disorder that results from a mutation in the *PIK3CA* gene, located on chromosome 3q26.32, which is present in the mosaic state. PROS encompasses a number of rare originally clinically defined conditions including acrocephaly-cutis marmorata telangiectasia congenita (MCAP), fibroadipose hyperplasia, CLOVE syndrome, Klippel-Trenaunay syndrome, hemimegalencephaly, and isolated lymphatic malformation [33–35, 64–69]. These conditions are characterized by segmental asymmetric overgrowth, vascular malformations, lymphatic malformations, lipomatous overgrowth, and skin manifestations such as epidermal nevi and have also been found to be associated infrequently with nephroblastomatosis and Wilms tumor [65, 70, 71].

6.2.3 Additional Wilms Tumor-Related Cancer Predisposition Syndromes

WT can occur as part of a cancer predisposition syndrome involving other well-defined genetic conditions such as seen with Fanconi anemia, Bloom syndrome, DICER1 syndrome, Li-Fraumeni syndrome, and mosaic variegated aneuploidy.

However, WT is usually not the main neoplasm associated with each of these syndromes.

6.2.3.1 Fanconi Anemia

Fanconi anemia is classified as a chromosomal breakage syndrome. It is typically characterized by short stature, radial ray defects, aplastic anemia/bone marrow failure, oral/head and neck cancers, acute myelogenous leukemia, and solid tumors of the head and neck, skin, gastrointestinal tract, and genitourinary tract [72]. There are approximately 21 different subtypes of Fanconi anemia, but there are two subgroups that are primarily characterized by risk for WT, medulloblastoma, and AML. Fanconi anemia subtype D1 (FANCD1) occurs from biallelic inheritance of mutations in the *BRCA2* gene located on chromosome 13q13.1, and Fanconi anemia subtype N (FANCN) arises from biallelic inheritance of mutations in the *PALB2* gene located on chromosome 16p12.2, which encodes a binding partner of BRCA2 [73–75]. Both are tumor suppressor genes. FANCD1 is associated with a 20% risk of WT [23, 74], whereas FANCN is associated with a 40% risk of WT [75]. Other subtypes of Fanconi anemia are not currently known to be associated with increased WT risk.

6.2.3.2 Bloom Syndrome

Bloom syndrome is a chromosomal breakage syndrome that is caused by biallelic inheritance of mutations in the *BLM* gene located on chromosome 15q26.1, which plays a role in chromosome stability [76]. This syndrome is characterized by growth deficiency including microcephaly, immune deficiency, photosensitivity, hyper- and hypopigmented skin findings, infertility in men, early menopause in women, insulin resistance, and the risk for a number of malignancies including leukemia; lymphoma; oropharyngeal, gastrointestinal, genitourinary, breast, skin, and lung cancers; and WT [77, 78]. Among the ~145 people in the Bloom syndrome registry as of 2018, 8% were reported as having WT with a mean age of 3 years [79].

6.2.3.3 Li-Fraumeni Syndrome

Li-Fraumeni syndrome (LFS) is an autosomal dominant cancer predisposition syndrome caused by heterozygous germline mutations in the tumor suppressor gene *TP53*, which is located on chromosome 17p13.1 [80]. Cancer risk varies by gender across the age spectrum. The cumulative cancer risk for female *TP53* mutation carriers is reported in one cohort to be 18%, 49%, 77%, and 93% by ages 20, 30, 40, and 50 years, respectively, whereas the cumulative risks for males is 10%, 21%, 33%, and 68% by the same ages [81, 82]. LFS is characterized by multiple cancers in one's lifetime. Some of the more common cancers include but are not limited to

early-onset breast cancer among females (frequently triple receptor positive), osteosarcoma less than 10 years of age, anaplastic rhabdomyosarcoma less than 3 years of age, adrenal cortical carcinoma, and brain tumors including choroid plexus carcinoma, high-grade glioma, diffuse nodular medulloblastoma (commonly the sonic hedgehog subtype), and hypodiploid ALL [82]. WT is not one of the classic cancers found in this syndrome but has been reported in families harboring *TP53* mutations and in several mutation-negative families that meet clinical criteria for LFS [83].

6.2.3.4 DICER1 Syndrome

Wilms tumor is infrequently associated with *DICER1* syndrome, also known as *DICER1*-related pleuropulmonary blastoma cancer predisposition syndrome [84–86]. This condition is autosomal dominantly inherited and caused by a mutation in the *DICER1* gene, which is located on chromosome 14q32.13 and is an RNase III-family endonuclease that cleaves precursor microRNAs (pre-miRNA) into active miRNA [84]. Approximately 80% of affected individuals inherit a mutation from a parent, while 20% of cases arise de novo. The *DICER1* mutation shows incomplete penetrance. The condition is characterized by pleuropulmonary blastoma usually prior to 6 years of age, cystic nephroma prior to 4 years of age, thyroid nodules and thyroid cancer over the life spectrum, ovarian Sertoli-Leydig cell tumors from childhood to the end of the female reproductive life cycle, nasal chondromesenchymal hamartoma, Wilms tumor prior to 5 years, botryoid rhabdomyosarcoma, pineoblastoma, pituitary blastoma, and a ciliary body medulloepithelioma [85]. There is a low risk of WT associated with most *DICER1* variants but a higher risk (18%) associated with the Gly803Arg variant [87, 88].

6.2.3.5 Mosaic Variegated Aneuploidy

There are three different types of mosaic variegated aneuploidy (MVA) including type 1 (associated with mutations in *BUB1B*), type II (associated with mutations in *CEP57*), and type 3 (associated with mutations in *TRIP13*). MVA1 is believed to be an autosomal recessive condition characterized by aneuploidy of multiple different chromosomes. The *BUB1B* gene is located on chromosome 15q15.1 and encodes one of the key proteins involved in the mitotic spindle checkpoint [53]. Patients with MVA1 can have a variable phenotype. Those who have been identified as having biallelic *BUB1B* mutations are more likely to present with growth retardation, mental retardation, and microcephaly and have shown an increased risk of rhabdomyosarcoma, whereas those with monoallelic *BUB1B* mutations with an unidentifiable second mutation have been characterized as having growth deficiency, mental retardation microcephaly, intrauterine growth retardation, cataracts, Dandy-Walker malformation, WT, and less commonly rhabdomyosarcoma. It is estimated that the risk of WT in individuals with MVA1 ranges from 25% to over 85% [89, 90], but *BUB1B* mutations are uncommon in sporadic WT [23]. MVA type 2 has not been reported

in association with WT, though WT has been reported among those with MVA type 3, which results from biallelic loss of function mutations in *TRIP13*, located on 5p15.33 [91]. Affected individuals are known to have microcephaly, developmental delay, seizures, café au lait spots and abnormal skin pigmentation, and WT. Biallelic *TRIP13* mutations are associated with a substantial impairment of spindle assembly checkpoint, which leads to chromosomal missegregation [91].

6.2.3.6 Mulibrey Nanism

Mulibrey nanism is an autosomal recessively inherited condition caused by mutations in the *TRIM37* gene located on chromosome 5p15.33 [92, 93]. This gene acts as a checkpoint regulator during cell division and ensures proper chromosome separation when cells divide [94, 95]. The condition is characterized by intrauterine growth retardation and postnatal failure to thrive, craniofacial features (scaphocephaly, facial triangularity, high and broad forehead, and low nasal bridge), perimyocardial heart disease/progressive cardiomyopathy, insulin resistance with type 2 diabetes, and additional features including a high-pitched voice, ocular findings including yellowish dots on ocular fundi, cutaneous naevi flammei, hepatomegaly, and fibrous dysplasia of long bones. Mild muscular hypotonicity has also been noted as has an increased frequency of respiratory infection [96]. Individuals with mulibrey nanism are at elevated risk for a variety of neoplasms. Females experience an increased risk for gynecological tumors including sex cord stromal tumors, ovarian adenofibroma, ovarian adenocarcinoma, and endometrial adenocarcinoma [97]. They also experience an increased frequency for premature ovarian failure and infertility. Additional tumors seen in association with this syndrome include thyroid cancer, gastrointestinal carcinoid tumor, neuro-pituitary Langerhans cell histiocytosis, acute lymphoblastic leukemia, liver tumors, and WT [96–98]. The risk to develop WT is about 4–6% [97, 98].

6.2.3.7 CDC73-Related Disorders

Mutations in the *CDC73* (*HRPT2*) gene, located on chromosome 1q31.2, show variable expressivity, and the phenotype may include (1) hyperparathyroidism-jaw tumor syndrome, (2) isolated parathyroid carcinoma, and (3) familial isolated hyperparathyroidism [99–102]. Hyperparathyroidism-jaw tumor syndrome is characterized by an increased risk for primary hyperparathyroidism due to parathyroid adenomas (95%) or parathyroid carcinoma (10–15%), ossifying fibromas of the mandible or maxilla (30–40%), malignant and benign uterine tumors, and renal lesions (20%) including cysts, hamartomas, and infrequently WT [9, 99, 103, 104]. More recently, isolated WT has been reported in association with mutations in *CDC73*, though the full spectrum of the disorder may not yet have manifested among this small number of affected individuals [9]. WT can result at a later age

including at least one individual who developed bilateral WT at 53 years of age [103, 104].

6.2.3.8 Bohring-Opitz Syndrome

Bohring-Opitz syndrome (BOS), which has previously been reported as Oberklaid-Danks syndrome, is a rare genetic syndrome caused by a mutation in the *ASLX1* gene located on chromosome 20q11.21. Cases to date have arisen de novo, and ~ 17 individuals reported in the medical literature have an identifiable mutation [105]. BOS is characterized by specific facial features (microcephaly and trigonocephaly, prominent metopic ridge, low tone, nevus flammeus, large/wide-set eyes, cleft palate, micrognathia), distinct posture (flexion at the elbows, wrists, and metacarpophalangeal joints), failure to thrive and feeding difficulties, seizures, severe cognitive impairment, limited mobility, mostly non-verbal, recurrent infections, congenital anomalies (including brain malformations, cardiac anomalies with possible bradycardia, and apnea), and severe myopia. Individuals with BOS have an increased but infrequent risk for WT (bilateral and nephroblastomatosis reported), and cases to date have been diagnosed from infancy to 6 years of age. The estimated risk of WT is ~7%, but this is based on a limited number of reported cases and larger studies are needed [105].

6.2.3.9 FBXW7-Related Wilms Tumor

FBXW7 is an autosomal dominant tumor suppresser gene located on chromosome 4q31.3 that has been found to predispose to WT [9, 106]. *FBXW7* mutations may contribute to a predisposition of a variety of tumors beyond WT. An individual with a *FBXW7* non-synonymous mutation and an extra-renal rhabdoid tumor and another individual with a WT and adult-onset osteosarcoma have been reported [9]. *FBXW7* deletion has been associated with a variety of tumors including adult WT, Hodgkin lymphoma, and breast cancer [9, 107]. Another individual with a translocation disrupting *FBXW7* (t(3:4)(q21;q31) has been described in an adult male with a history of a renal cell carcinoma [9, 108].

6.2.3.10 KDM3B-Related Wilms Tumor

The *KDM3B* gene is located on 5q31.2, and cancer-predisposing mutations are inherited in an autosomal dominant manner. Mutations have been described in association with WT, hepatoblastoma, acute myeloid leukemia, and Hodgkin lymphoma [9, 109]. Affected individuals have also been reported to have non-cancerous medical features including hyper- and hypopigmented macules, hip dysplasia, autism, and intellectual disabilities. The spectrum of medical issues associated with

mutations in *KDM3B* needs further delineation. Pathogenic mutations have been described as both non-synonymous and truncating mutations.

6.3 Non-syndromic Wilms Tumor

6.3.1 Non-syndromic WT1-Related Wilms Tumor

Some individuals with a *WT1* mutation only develop WT without the syndrome-related manifestations of Denys-Drash, Frasier, or WAGR syndromes. Hence, affected individuals can display an "incomplete" phenotype. The chance of isolated Wilms tumors arises from a *WT1* mutation in about 5% [16, 110, 111].

6.3.2 TRIM28-Related Wilms Tumor

TRIM28 is a tumor suppressor gene that plays a role in DNA repair and maintenance of genomic integrity. It is located on chromosome 19q13.4 and has been implicated in pathogenicity of WT [9, 112, 113]. Both familial and de novo pathogenic truncating and less frequently missense *TRIM28* mutations have been identified in individuals with WT. Other childhood and adult cancers have not been found in association with this gene, suggesting that mutations in *TRIM28* predominantly predispose to both unilateral and bilateral WTs. One individual with a *TRIM28* mutation and WT has also been reported to have esophageal atresia and a heart defect, though it is not clear if *TRIM28* played a role in the development of these malformations [113], and 2 of 21 individuals (both males) with *TRIM28* mutations and WT were reported to have autism and delays [9]. *TRIM28* mutations show incomplete penetrance. Those resulting in WT have been significantly associated with maternal inheritance of the pathogenic allele. Hence, there appears to be a parent of origin effect [9, 113]. Histology among *TRIM28*-related WT is predominantly epithelial, and nephroblastomatosis has been reported [9, 112–114].

6.3.3 NYNRIN-Related Wilms Tumor

NYNRIN-truncating mutations inherited in an autosomal recessive manner have been reported in association with WT [9]. Little is known about the *NYNRIN* gene, which is located on chromosome 14q12, but it is believed to be play a role in microRNA processing (Peng et al., 2018). Mutations in this gene are not known to be associated with other childhood or adult-onset tumors, though the number of affected individuals reported in the medical literature is limited [9].

6.3.4 REST-Related Wilms Tumor

REST is located on chromosome 4q12 and is a dominantly inherited tumor suppressor gene in which mutations in the DNA-binding domain affect transcription. This gene accounts for approximately 2% of WT development, and both familial and non-familial cases have been reported. While *REST* plays a role in cellular differentiation and embryonic development, no medical problems of statistical significance have been associated with mutations in *REST* beyond WT [115].

6.3.5 CTR9-Related Wilms Tumor

CTR9 is a part of the polymerase-associated factor 1 complex, which resides on chromosome 11p15.4 and plays a role in RNA polymerase II regulation. It is important in embryonic organogenesis and maintenance of embryonic stem cell pluripotency [116]. *CTR9* has been implicated as a tumor suppressor gene that contributes to the pathogenesis of Wilms tumor [116, 117]. It has not been found in association with other tumors or syndromic features. While there have been several genes identified in recent years that have been found in association with familial WT, some families that exhibit a familial pattern of WT inheritance have no identifiable gene mutation suggesting that there remain undiscovered familial WT genes [9, 75].

6.4 Surveillance

Surveillance imaging is recommended for individuals with a genetic predisposition to WT [31, 118, 119]. The principle of surveillance is to enable detection of WT at a small size, resulting in lower tumor stage, though there is limited evidence-based data that surveillance results in improved WT-related outcomes. Practical surveillance recommendations have been developed based on the premise that surveillance is worthwhile in individuals who have greater than a 1% [119] or 5% risk of developing WT [31, 118]. Most expert recommendations are for surveillance to continue until the age when about 90–95% of the WT will have occurred; therefore, the duration of surveillance varies according to the genetic disorder and how conservative the provider and family wish to be (Table 6.2). For *WT1*-related disorders, 94% of WT occur by the age of 5 years and 98% occur by 6 years [120]. For the BWS spectrum, 93% of WT occur by 7 years, 95% occur by 8 years, and 96% occur by 9 years. For most of the other conditions, the risk period is not well-defined, and various surveillance periods have been recommended (Table 6.2). For individuals who are selected to undergo surveillance, the recommended procedure is renal ultrasonography every 3 months. As WT can have a doubling time of 7–21 days, this frequency of screening optimizes the chance of detecting a tumor when it is a small

size [121]. Genetic counseling should be performed when concern is raised for a cancer predisposition syndrome both prior to and following genetic testing [122].

6.5 Summary and Future Directions

Our understanding of WT genetics has evolved considerably since *WT1* was described in 1990. In contrast to the situation with retinoblastoma, where *RB* gene mutations are the primary event in the vast majority of tumors, WT may arise through several distinct genetic pathways. Somatic mutations in *WT1*, *AMER1*, and *CTNNB1* together provide the genetic basis for about one-third of all WT. Other common somatic mutations involve miRNA processing genes, accounting for approximately 15% of WT. Genetic and epigenetic alterations of the 11p15/*IGF2* locus are seen in more than 70% of WT, though these alterations are not thought to be sufficient for Wilms tumorigenesis. Somatic *TP53* mutations are observed in most anaplastic histology WT but are rare in tumors without anaplasia. The most commonly observed genes with constitutional mutations in patients with WT are *WT1*, *TRIM28*, *IGF2*, *REST*, and *CTR9*, though constitutional mutations have been described in more than 16 other genes. Despite the recent tremendous expansion of knowledge, the genetic basis of many WTs remains unaccounted for. Ongoing comprehensive genomic analyses, including whole-genome sequencing and gene expression, methylation, microRNA expression, and single-nucleotide polymorphism arrays will likely elucidate additional genetic lesions that drive Wilms tumorigenesis or modify its clinical behavior.

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Chapter 7
Hereditary Overgrowth Syndromes

Jack Brzezinski, Cheryl Shuman, and Rosanna Weksberg

Abstract In this chapter, we review a number of overgrowth syndromes with respect to clinical features including tumor risk and the molecular testing approaches. The concepts of imprinting and epigenetically driven disease are introduced along with the molecular networks that are shared by constitutional overgrowth and tumor development. We outline the importance of precision for clinical and molecular diagnosis for all children with overgrowth syndromes using a combination of modalities where indicated. Clinicians are encouraged to consider, for any child presenting with a tumor, the growth trajectory and other clinical findings to facilitate a diagnosis of an overgrowth syndrome that confers an increased risk for tumor development.

Keywords Epigenetics · Overgrowth · Uniparental disomy · Renal tumors

7.1 Introduction

For children who present with overgrowth, accurate clinical and molecular diagnosis is critical for estimating their risk for tumor development. Overgrowth syndromes are defined as conditions which demonstrate either generalized or localized excess growth when compared to standardized growth parameters for age and sex.

J. Brzezinski
Division of Haemotology/Oncology, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada

Department of Pediatrics, University of Toronto, Toronto, ON, Canada

C. Shuman
Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada

R. Weksberg (✉)
Department of Pediatrics, University of Toronto, Toronto, ON, Canada

Department of Clinical and Metabolic Genetics, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada
e-mail: rweksb@sickkids.ca

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A number of genetic syndromes associated with overgrowth in childhood have been well described [1]. Examples of syndromes associated with generalized overgrowth (i.e., height, weight, and/or head circumference above the 97th percentile for sex and age) include Beckwith-Wiedemann, Sotos, Perlman, Simpson-Golabi-Behmel, and Weaver syndromes. Isolated hemihyperplasia, Klippel-Trenaunay, and Proteus syndromes are examples of overgrowth syndromes involving localized or asymmetric overgrowth.

A predisposition to pediatric malignancies has long been recognized for some overgrowth syndromes. For some of these syndromes, the spectrum of tumor types and their frequencies has been used to develop tumor screening protocols. For others, such as Sotos syndrome, the frequencies and range of tumor types have not supported targeted tumor screening protocols. Finally, for some overgrowth syndromes, such as Weaver syndrome, currently available data are not conclusive in determining whether a tumor surveillance program is indicated [2]. Notably, overgrowth syndromes are often diagnosed only when a child presents with a tumor. This may be due to subtle clinical features or to the lack of recognition of a specific constellation of features. Therefore, clinicians should not only be aware of tumor risk in children with known overgrowth syndromes, but should also be aware of potential overgrowth syndromes in children presenting with specific tumors. This requires gathering data on syndromic clinical features, as well as all growth parameters, including head circumference and birth weight.

In this chapter, we review a number of overgrowth syndromes associated with increased risks of malignant tumors, beginning with Beckwith-Wiedemann syndrome and isolated hemihyperplasia. We will also address relevant molecular etiologies including epigenetic regulation and imprinting, recurrence risks, spectrum and frequencies of associated tumor types, and the currently recommended screening protocols. A summary of the overgrowth syndromes reviewed here is presented in Table 7.1.

7.2 Epigenetics and Imprinting

To date, the most commonly diagnosed overgrowth disorders have been Beckwith-Wiedemann and Sotos syndromes [1]. The molecular etiologies of these two conditions demonstrate two different types of epigenetic dysregulation.

Children with Beckwith-Wiedemann syndrome have alterations in the epigenetic marks at a specific genomic region. Children with Sotos syndrome have loss of function mutations in a gene that encodes a protein involved in the physiologic modifications of the epigenome. In normal human growth and development, the regulation of gene expression is tightly programmed by epigenetic marks, including DNA methylation at cytosine residues in CpG dinucleotides, modifications to histone proteins, non-coding RNAs, and chromatin conformation. These marks, which are extrinsic to DNA sequence, guide specific cell type, tissue, and organ differentiation by allowing the transcriptional machinery to gain access to critical regions of

Table 7.1 Summary of overgrowth syndromes' known molecular mechanisms, tumors at risk, and suggested screening protocols [11, 25, 79, 81, 88, 91, 106–111]

	Molecular mechanism	Locus	Common tumors reported	Less commonly reported tumors	Screening protocol
Beckwith-Wiedemann syndrome	See Table 7.2	11p15	Wilms tumor, hepatoblastoma, rhabdomyosarcoma, neuroblastoma, adrenocortical carcinoma	Hepatocellular carcinoma, gonadoblastoma, pheochromocytoma, thyroid carcinoma, glioblastoma, lymphoma, pancreatoblastoma, carcinoid tumor, congenital mesoblastic nephroma, renal cell carcinoma, yolk sac tumor, intratubular germ cell neoplasm, adrenal adenoma, fibroadenoma	Serum AFP q3 months for 4 years Abdominal US q3 months for 8 years
Isolated hemihyperplasia	See Table 7.2	11p15	Wilms tumor, hepatoblastoma, rhabdomyosarcoma, neuroblastoma, adrenocortical carcinoma	Pheochromocytoma, testicular carcinoma, undifferentiated sarcoma, breast cancer, adrenal adenoma	Same as for Beckwith-Wiedemann syndrome
Mosaic genome-wide uniparental disomy	Mosaic genome-wide uniparental disomy	Multiple	Wilms tumor Hamartoma	Pheochromocytoma	Same as for Beckwith-Wiedemann syndrome
Perlman syndrome	*DIS3L2*	2q37	Wilms tumor		No established protocols; frequent clinical exams and abdominal ultrasounds recommended from birth

(continued)

Table 7.1 (continued)

	Molecular mechanism	Locus	Common tumors reported	Less commonly reported tumors	Screening protocol
Weaver syndrome	*EZH2*	7q36	Neuroblastoma, lymphoma, acute lymphoblastic leukemia		Not yet defined
Sotos syndrome	*NSD1*	5q35	Acute lymphocytic/ lymphoblastic leukemia, non-Hodgkin's lymphoma	Wilms tumor, hepatocellular carcinoma, gastric adenocarcinoma, vaginal epidermoid carcinoma, small cell carcinoma of the lung, hepatoblastoma, sacrococcygeal teratoma	Screening protocol not currently in use
Simpson-Golabi-Behmel syndrome	*GPC3*	Xq26	Wilms tumor, hepatoblastoma, hepatocellular carcinoma	Neuroblastoma, gonadoblastoma, medulloblastoma, ameloblastoma	Same as for Beckwith-Wiedemann syndrome

DNA at precise times during embryonic and fetal development. Epigenetic marks are stably heritable but also dynamic in that they can change in response to environmental influences. Disruption of normal epigenetic patterns can lead to disorders that present with overgrowth, sometimes in association with cancer, and/or intellectual disability.

Epigenetic marks are regulated by a variety of proteins that have precise functions. These proteins – often referred to as the cell's "epigenetic machinery" – attach, erase, or read specific epigenetic marks and define regional chromatin conformation and transcriptional activity. Loss-of-function genomic variants in the genes that encode these proteins result in overgrowth disorders such as Sotos syndrome and in sporadic cancers.

Unlike Sotos syndrome, Beckwith-Wiedemann syndrome is often driven directly by modifications of normal epigenetic imprinting at specific genomic regions. Imprinting refers to the epigenetically regulated differential, monoallelic expression of genes in a parent of origin-specific manner. That is, either the paternal allele or the maternal allele is preferentially or exclusively expressed. This parent of origin-specific allelic expression is regulated by layered epigenetic marks including DNA methylation and histone modifications. Usually, but not always, the methylated allele is the one that is silenced [3]. It is estimated that there are currently approximately 86 imprinted autosomal genes in humans (http://otago.ac.nz/IGC). Many of these genes are critical for normal embryonic growth and neurodevelopment [4]. Thus, it is not surprising that a number of different growth and neurodevelopmental disorders are caused by epigenetic (and genetic) alterations that compromise the expression of imprinted genes [5, 6]. Most imprinted genes are located in clusters within imprinted domains. The transcription of imprinted genes in these domains is usually regulated *in cis (*i.e., along the same chromosome) over large genomic distances (>1 Mb) by imprinting centers (ICs). These ICs are also called differentially methylated regions (DMRs) because the parental chromosomes exhibit opposite methylation patterns. Genomic and/or epigenomic alterations that change such patterns of DNA methylation at imprinting centers and/or imprinted genes can cause overgrowth disorders such as Beckwith-Wiedemann syndrome.

7.3 Beckwith-Wiedemann Syndrome and Isolated Hemihyperplasia

Beckwith-Wiedemann syndrome is a heterogeneous condition, both in clinical presentation and molecular etiology. It has an estimated prevalence of 1 in 10,000 – 20,000 live births and affects males and females equally [7–9]. This prevalence likely represents an underestimate, as milder phenotypes may not be ascertained. Children with Beckwith-Wiedemann syndrome often present with generalized overgrowth with height and weight plotting above the 97th percentile but head circumference plotting around the 50th percentile. However, factors such

as prematurity and parental heights may impact presenting growth parameters and should be considered in the initial assessment. Overgrowth may also be manifested regionally as hemihyperplasia (aka lateralized overgrowth) which is defined as ipsilateral or contralateral asymmetric overgrowth of one or more body parts. Notably, hemihyperplasia can be present in the context of Beckwith-Wiedemann or other syndromes or in isolation (isolated hemihyperplasia) (see below). The facial gestalt may include prominent eyes with infraorbital creases, facial nevus flammeus, midfacial hypoplasia, macroglossia, full lower face with a prominent mandible, anterior earlobe creases, and posterior helical pits (Fig. 7.1). For Beckwith-Wiedemann syndrome, additional common clinical findings include neonatal hypoglycemia, abdominal wall defects (omphalocele, umbilical hernia, diastasis recti), visceromegaly involving a single organ or any combination of abdominal organs (liver, spleen, pancreas, and kidneys), fetal adrenocortical cytomegaly, and embryonal tumors (see below). Several non-malignant renal abnormalities have also been described such as medullary dysplasia, nephrocalcinosis, nephrolithiasis, and medullary sponge kidney disease [10, 11].

Clinical features tend to normalize across childhood making clinical diagnosis in older children more challenging. Thus, a thorough review of early childhood findings including photographs is recommended. Intellectual development is usually normal in the absence of a chromosome abnormality (see below), abnormality of the posterior fossa [12]), or untreated neonatal hypoglycemia [13].

It is well accepted that Beckwith-Wiedemann syndrome is associated with the development of embryonal tumors. The majority of the risk for tumor development occurs in the first 8 years of life and is estimated to be 7.5% ranging in different studies from 3.4% to 21% [14–23]. Wilms tumor is the most common neoplasia observed in patients with Beckwith-Wiedemann syndrome followed by hepatoblastoma and, more rarely, rhabdomyosarcoma, adrenocortical carcinoma, and neuroblastoma. Additional tumors reported in individuals clinically diagnosed with Beckwith-Wiedemann syndrome are noted in Table 7.2. Wilms tumors tend to occur at a younger age in children with Beckwith-Wiedemann syndrome and are more likely to be bilateral compared to children who develop sporadic Wilms tumor.

Isolated hemihyperplasia in the absence of other clinical findings indicative of a syndromic diagnosis also carries a risk of approximately 6% for developing an overlapping spectrum of embryonal tumors as is seen in children with Beckwith-Wiedemann syndrome [24, 25] (Table 7.1). Clinically, it is important to distinguish hemihyperplasia from hemihypoplasia when evaluating body asymmetry as hemihypoplasia is not known to be associated with an increased risk for tumor development [26].

Molecular alterations in an imprinted genomic region of chromosome 11p15.5 have been associated with Beckwith-Wiedemann syndrome, isolated hemihyperplasia, and an increased risk for the development of Wilms and other tumors [15, 27–30]. Although both Beckwith-Wiedemann syndrome and isolated hemihyperplasia are diagnosed clinically, molecular diagnostics are important for several reasons: (1) specific types of genetic and epigenetic abnormalities are correlated with tumor risk, (2) risk of recurrence of these conditions in future children within a family is

Fig. 7.1 Child with Beckwith-Wiedemann syndrome

Table 7.2 Stratification of Beckwith-Wiedemann syndrome patients based on molecular abnormality and risk of tumor development

Group	Molecular abnormality	Percentage (%) of patients with BWS	Risk of tumor development	Types of tumors
1	UPD 11p15 (gain of methylation IC1 and loss of methylation IC2)	20%	High (15–20%)	Wilms tumor, hepatoblastoma, neuroblastoma, and adrenocortical carcinoma
2[a]	Gain of methylation IC1	5%	High (>30%)	Wilms tumor
3[a]	Loss of methylation IC2	50%	Low (<5%)	Hepatoblastoma, rhabdomyosarcoma, Wilms tumor
4	Pathogenic variant in the *CDKN1C* gene	5%	Low to moderate (~8%)	Neuroblastoma, case reports of other tumor types
5	Cytogenetically visible duplications, translocations/inversions of 11p15.5	1–2%	Very low	Case reports of adrenal tumor and Wilms tumor
6	Not identified	10–30%	High (10–20%)	Wilms tumor

[a]May be associated with genomic microdeletions or microduplications

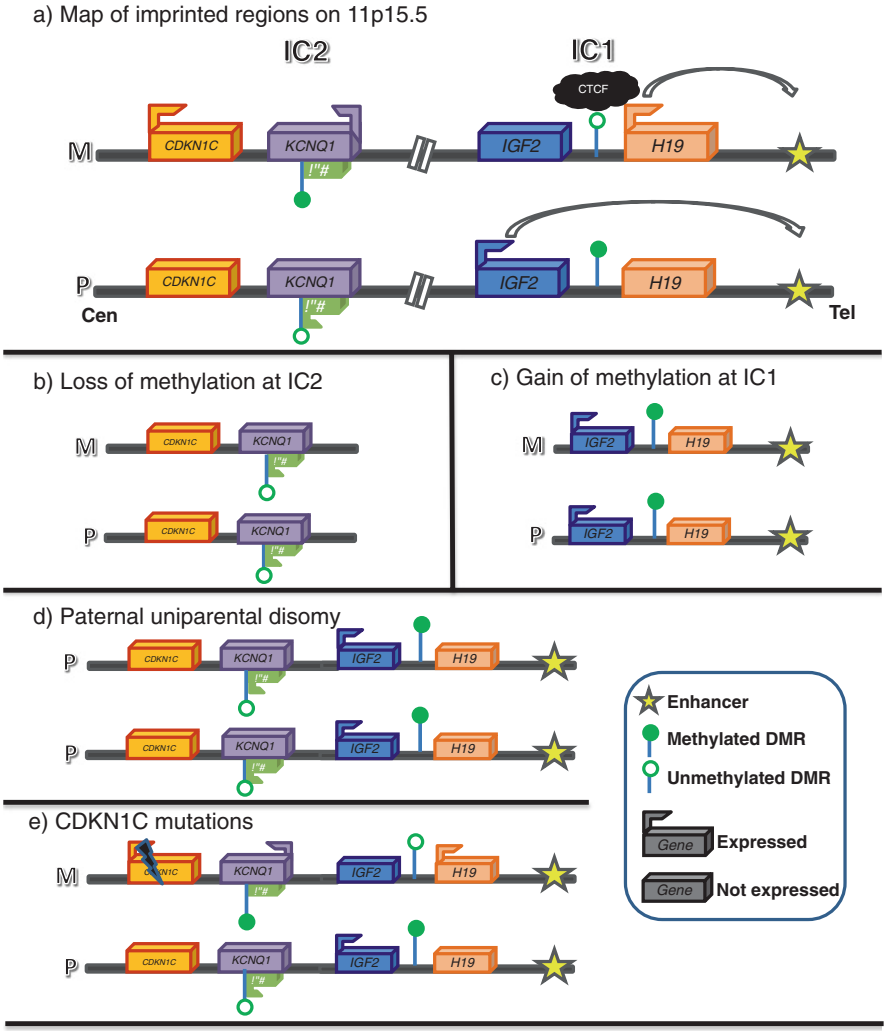

Fig. 7.2 Schematic of the imprinting centres on chromosome 11p15.5 (**a**) and the pathogenic alterations that can occur in the region in children with Beckwith Wiedemann syndrome (**b-e**)

dependent on the molecular diagnosis, and (3) additional non-malignant phenotypic features of Beckwith-Wiedemann syndrome are correlated with specific alterations at chromosome 11p15.5 [14, 22, 23].

Two imprinted domains have been identified on chromosome 11p15, each encompassing a cluster of imprinted genes (Fig. 7.2). Within each domain an imprinting center (IC) regulates the imprinted genes via differential methylation of the two alleles. IC1 regulates the expression of two imprinted genes: *IGF2* (insulin-like growth factor 2), which is usually paternally expressed, and *H19*, a noncoding

RNA that is maternally expressed. Gain of methylation (GOM) at IC1 is seen in 5–9% of Beckwith-Wiedemann syndrome cases and leads to biallelic expression (i.e., expression from both the paternal and maternal alleles) of *IGF2* [31]. IC2 overlaps the promoter of a long non-coding RNA transcript, KCNQ1OT1, and regulates *in cis* the expression of several maternally expressed imprinted genes in this domain, most importantly *KCNQ1OT1* and *CDKN1C*. *KCNQ1OT1* is a paternally expressed noncoding RNA transcript that regulates *in cis* the expression of the maternally expressed imprinted genes in this domain. The *CDKN1C* gene encodes the $p57^{KIP2}$ protein, a member of the cyclin-dependent kinase inhibitor gene family, which negatively regulates cell proliferation. It is both a tumor suppressor gene and a negative regulator of fetal growth. IC2 is normally methylated on the maternal chromosome blocking the expression of *KCNQ1OT1* and permitting *CDKN1C* to be expressed from the maternal allele. Loss of methylation (LOM) at IC2, seen in 40–60% of individuals with Beckwith-Wiedemann syndrome, is associated with expression of *KCNQ1OT1* on the maternally derived chromosome and silencing of *CDKN1C*. A third category of Beckwith-Wiedemann syndrome cases demonstrates epigenetic alterations involving both IC1 (GOM) and IC2 (LOM), a pattern which is usually indicative of paternal uniparental disomy (UPD), i.e., the presence of two chromosomal regions from the father and none from the mother. UPD is detected in approximately 20% of individuals with Beckwith-Wiedemann syndrome.

Approximately 85% of Beckwith-Wiedemann syndrome cases are sporadic, have a normal karyotype, and carry one of the epigenetic disruptions mentioned above [13]. However, genetic changes at 11p15 can also lead to Beckwith-Wiedemann syndrome, and these include pathogenic variants in the *CDKN1C* gene (approximately 5% of non-familial cases and ~ 40% when with a positive family history), chromosome inversions, translocations, and duplications (~1–2% of cases), and microdeletions or microduplications involving one or more of the critical genes on chromosome 11p15.5 [32].

From a molecular standpoint, Beckwith-Wiedemann syndrome can be divided into six groups, and each group appears to have a distinct tumor predisposition profile, both in terms of tumor frequency and tumor type (Table 7.2) [1, 15, 18]. Group 1 is represented by UPD and is associated with development of Wilms tumor, hepatoblastoma, neuroblastoma, and/or adrenocortical carcinoma. The risk in this group for developing these tumors is ~16% [15, 18]. Group 2 is represented by GOM at IC1 due to an imprinting alteration which may or may not be associated with an underlying genomic abnormality (i.e., microdeletion or microduplication); this group has the greatest risk for developing tumors, on the order of >30% [15, 18]. The tumors described in group 2 are predominantly composed of Wilms tumor (~85%). Patients in group 3 show LOM at IC2; genomic abnormalities in this group are rare. Group 3 seems to carry a much lower risk for tumor development (2–3%) [15, 18]. Tumors in patients with this epigenetic error mostly include hepatoblastoma and rhabdomyosarcoma. Wilms tumors are rare in this group but have been reported by several authors [16, 18, 33–36]. Group 4 is characterized by pathogenic variants in the *CDKN1C* gene. These children have up to 8% risk of tumor development, 75% of which are neuroblastoma. The other tumors reported in this group are

all single cases and include leukemia, melanoma, and soft tissue tumors. Group 5 is characterized by chromosomal abnormalities visible on karyotype including duplications (paternal chromosome), translocations, or inversions (maternal chromosome). Such findings are detected in ~1–2% of cases of Beckwith-Wiedemann syndrome. The risk for tumor development in this group is very low; a single case of adrenal tumor has been reported along with one child who presented sequentially with diffuse hyperplastic nephrogenic rests, Wilms tumor, and hepatoblastoma [37, 38]. Finally, Group 6 demonstrates normal imprinting (normal methylation patterns in IC1 and IC2) and no genomic alterations. The number of children in this category ranges from 10% to 30% in different case series and carries an approximately 10–20% risk of developing embryonal tumors, including Wilms tumor [8, 15, 18, 39]. The variability and tumor risk may reflect somatic mosaicism for chromosome 11p15 UPD at a concentration that is below the level of detection in the tissue sampled (e.g., blood). The level of detection varies by the testing modality and the number of different tissues tested [40–42]. Group 6 may also include other molecular mechanisms as yet undescribed but potentially associated with tumor development in Beckwith-Wiedemann syndrome.

Beckwith-Wiedemann syndrome is associated with an increased incidence of monozygotic twins. Most commonly, these twins are female and discordant for Beckwith-Wiedemann syndrome. As well, they are usually discordant for loss of methylation at IC2 in skin fibroblasts but variably concordant in blood cells, probably as a result of shared fetal circulation [43]. Male monozygotic twins are also reported but at a much lower frequency than females. Male twins demonstrate a range of molecular etiologies parallel to that seen in singleton Beckwith-Wiedemann syndrome cases including loss of methylation at IC2, UPD for 11p15, and gain of methylation at IC1 [44]. Tumor surveillance is suggested for the apparently unaffected monozygotic twin as shared fetal circulation occurs often in monozygotic twinning. In addition, monozygotic twins may carry unequal levels of mosaicism of chromosome 11p15.5 alterations, e.g., UPD, so that one appears clinically affected, while the other does not, when in fact both may carry a tumor predisposing molecular alteration in the liver or kidney. To date, there have been no published reports of tumor development in the phenotypically milder or unaffected twin.

Intriguingly, children conceived using assisted reproductive technologies appear to be at increased risk of Beckwith-Wiedemann syndrome with an enrichment of loss of methylation at IC2 which is reported in 95% of the cases [13]. It has been suggested that parental subfertility and/or the use of reproductive technologies may increase the risk of imprinting disorders due to errors either in the establishment or maintenance of imprints in the sperm, egg, or early embryo, but further evidence is required to support this theory [45].

The risk of recurrence to parents of a child with Beckwith-Wiedemann syndrome in future pregnancies as well as transmission to offspring from an individual with this disorder is contingent upon the molecular alteration(s) involved. Patients who have a documented genomic alteration have an increased risk for recurrence usually following an autosomal dominant pattern (50% chance); however, this risk is also dependent on the sex of the transmitting parent [13]. In contrast, individuals with

exclusively epigenetic changes do not generally appear to have a significant risk for transmission as these epigenetic alterations are usually reprogrammed during gametogenesis. This low risk also applies to the recurrence risk for parents of a child with an exclusively epigenetic alteration. In the case of UPD for chromosome 11p15.5, this finding is believed to be the consequence of a post-zygotic recombination error and is inferred to be an early lethal abnormality as it consistently demonstrates somatic mosaicism; that is, it is present at variable levels across multiple tissues of affected individuals [13, 27]. Since gametes are haploid and epigenetic marks are reprogrammed during gametogenesis, UPD is not expected to be transmissible. As well, the mechanism of post-zygotic recombination suggests that parents of a child with UPD for chromosome 11p15.5 would not have a significantly increased risk for recurrence. In contrast, GOM at IC1 can be the consequence of a (potentially heritable) microdeletion or of an isolated and usually sporadic epigenetic alteration. These data underscore the importance of a comprehensive molecular analysis for every patient with suspected Beckwith-Wiedemann syndrome with a view to providing accurate genetic counseling [13, 46].

Although many patients with isolated hemihyperplasia do not have an identifiable molecular abnormality using currently available diagnostic methodologies [24, 28], a significant proportion of cases are driven by the same alterations at chromosome 11p15.5 as are seen in Beckwith-Wiedemann syndrome. In fact, UPD for chromosome 11p15.5, GOM at IC1, and LOM at IC2 have all been identified in children with isolated hemihyperplasia [24, 28, 47]. The lower level of detection is likely due to somatic (post-zygotic) mosaicism for the molecular alterations leading to overgrowth [13, 28] below the level of detection in the tissue tested.

Many centers recommend that patients with Beckwith-Wiedemann syndrome and isolated hemihyperplasia be screened for abdominal embryonal tumors regardless of whether or not a molecular alteration has been detected [16, 24, 48, 49]. We do not recommend that tumor surveillance protocols be modified based on molecular stratification at this time, as more data are required to generate evidence-based guidelines. However, the literature on this issue continues to evolve both in terms of who should undergo surveillance and how surveillance should be undertaken. We discuss our current recommendations for tumor surveillance below, but readers should be aware that jurisdictional differences may exist.

The current tumor surveillance protocol widely adopted across North America includes abdominal ultrasound every 3 months until 8 years of age [50–52]. Hepatoblastoma typically presents prior to the age of 4 years; therefore AFP (alpha-fetoprotein) levels should be checked every 3 months until 4 years of age.

A review of published reports indicates that the median age of hepatoblastoma diagnosis in the Beckwith-Wiedemann syndrome population is 5 months of age compared to 16 months in those who developed the tumor sporadically. Furthermore, 97% of cases were diagnosed before 24 months of age [53]. These findings have led to a suggestion that only children <24 months be screened by AFP measurement. When considering this suggestion, we must take into account the retrospective and selective nature of the majority of reports included in this review and understand that some children with milder phenotypes of Beckwith- Wiedemann syndrome

may have been classified as sporadic cases. We know of at least one case diagnosed after 24 months of age not included in the Mussa review [54]. A large-scale prospective analysis including children with milder manifestations of Beckwith-Wiedemann syndrome may produce different results. As well, although liver masses can be detected by ultrasonography, AFP is a sensitive and specific tumor marker that may be elevated before a mass grows large enough to be detected on screening ultrasound. In these cases, timely cross-sectional imaging can detect a small tumor. As hepatoblastoma outcomes depend greatly on the proportion of involved liver, early detection likely leads to improved survival and, in some cases, avoidance of adjuvant chemotherapy [55]. For these reasons, we continue to recommend quarterly AFP screening until 4 years of age while we await development of a broader evidence base on this issue.

Based on the reported low incidence of tumors in children with Beckwith-Wiedemann syndrome in group 3 with IC2 LOM, a consensus statement was issued for this group of children living in Europe and the UK, maintaining that tumor surveillance should not be undertaken for these children after an initial baseline ultrasound. In North America, however, routine tumor surveillance continues to be offered for this group of children for several reasons. Firstly, as discussed above, accurate molecular classification is dependent both on assay sensitivity and on the tissue tested, and there are reported cases of children with paternal uniparental disomy who were misclassified as IC2 LOM in experienced labs. Secondly, in the past several years, an increasing number of children classified with IC2 LOM have been reported to develop Wilms tumor. To our knowledge, at the time of writing, 12 such cases are reported in the literature, and the number will likely continue to increase [21, 23, 33–35, 56, 57]. Thirdly, the incidence of abdominal tumors (hepatoblastoma and Wilms tumor) has been reported to be up to 3% in this group. Even this low incidence compared to the general Beckwith-Wiedemann syndrome population is still an 11-fold increase from the general population [35]. Finally, the benefits of screening likely outweigh the risks. Reviews of Wilms tumor cases in North America enrolled in the National Wilms Tumor Study trials indicate that surveillance is associated with lower tumor stage at diagnosis [58]. Diagnosis at a lower stage allows both for a reduction of chemoradiotherapy and a higher likelihood of successful nephron-sparing surgery—crucial in a group of children at risk for other forms of renal disease [59, 60]. Although some clinicians report an increase in anxiety with surveillance imaging, interviews with families indicate that the anxiety arises primarily from the underlying tumor risk and not the surveillance program per se [61]. Ultrasonography as a modality carries little risk, can be done without sedation, and involves minimal discomfort.

Counseling of families should include the fact that the screening protocol does not capture all tumors either in terms of organs involved (e.g., rhabdomyosarcoma) or timeframe. The surveillance protocol targets the most frequently reported tumors which are found in the abdomen and the most common ages at presentation (<8 years). Wilms tumors and hepatoblastoma have been infrequently reported after age 8 years in Beckwith-Wiedemann syndrome individuals, and there are a number of rare tumors which can present outside the abdomen including

rhabdomyosarcoma and thoracic neuroblastic tumors (Table 7.2) [54, 62]. Therefore, any suspicious clinical concerns should be thoroughly investigated regardless of the tissue involved or the age of the individual with Beckwith-Wiedemann syndrome.

7.4 Chromosome 11p15.5 Alterations in Wilms and Other Embryonal Tumors

It has long been known that children with Beckwith-Wiedemann syndrome are more likely to harbor nephrogenic rests in their kidneys than children with sporadic Wilms tumors. The presence of these premalignant lesions suggest a "field defect" in the kidneys; that is, many parts of the tissue are primed to develop Wilms tumors [63]. These findings, along with the increased preponderance of bilateral tumors in syndromic children, led the Children's Oncology Group to trial a unique approach to Wilms tumor treatment in children with Beckwith-Wiedemann syndrome. Although upfront total nephrectomy followed by adjuvant chemoradiotherapy is the standard of care for bilateral disease, the AREN0534 trial used neoadjuvant therapy in an attempt to facilitate nephron-sparing surgery. Recently presented data from this trial are very encouraging in that the results show that oncologic outcomes can be improved by taking predisposition syndromes into account [64].

There is a clear association between Wilms tumors and the loci involved in Beckwith-Wiedemann syndrome. Epigenetic errors in chromosome 11p15 have been demonstrated in multiple somatic tissues of up to 70% of children who develop "sporadic" Wilms tumors, i.e., in the absence of a syndromic diagnosis such as Beckwith-Wiedemann syndrome or isolated hemihyperplasia [65–68]. In these Wilms tumor cases, the epigenetic change often involves IC1, either through gain of methylation or paternal uniparental disomy. Even more striking is the nearly universal overexpression of *IGF2* in up to 90% of Wilms tumors [69]. Finally, recent work has demonstrated that in a proportion of WT, the first molecular event to occur is gain of methylation at IC1 found sometimes even in histologically normal kidney surrounding the tumor [70]. These findings highlight the critical role of the expression of genes on chromosome 11p15.5 in Wilms tumor development and the molecular connection between these tumors and Beckwith-Wiedemann syndrome. These presence of such molecular changes are also important for therapeutic considerations. Somatic epigenetic changes at chromosome 11p15.5 are a predictor of treatment failure in the small subset of infants with stage I Wilms tumors that could otherwise be cured by surgery alone [71].

Chromosome 11p15.5 epigenetic changes have been seen in the blood of up to 3% of individuals with Wilms tumor who are described to be non-syndromic. This occurs especially in cases of bilateral tumors [27] demonstrating perhaps a very mild phenotype of Beckwith-Wiedemann syndrome with somatic mosaicism. These findings demonstrate the importance of considering overgrowth syndromes in all children presenting with Wilms tumors and carefully evaluating each child with

careful history and physical examination including assessment of growth parameters and targeted molecular testing.

The occurrence of somatic 11p15 epigenetic changes has also been demonstrated in about 20–30% of hepatoblastomas [30, 72] and is prevalent in embryonal rhabdomyosarcoma although a precise frequency is yet to be determined [73]. In conclusion, further elucidation of the molecular basis of Beckwith-Wiedemann syndrome and isolated hemihyperplasia is needed not only to guide counseling regarding the risk for tumor development and tumor surveillance strategies but also to address prognosis and potentially even treatment approaches for both syndromic and non-syndromic embryonal tumors [60].

7.5 Genome-Wide Paternal Uniparental Disomy (UPD)

A new overgrowth syndrome caused by somatic mosaicism for paternal UPD of all chromosomes has recently been described in a small number of case reports and case series. Children with this syndrome have features defined by dysregulation at multiple imprinted loci. Clinical features can include those seen in Beckwith-Wiedemann syndrome, such as overgrowth, hemihyperplasia, and macroglossia associated with mosaicism for 11p15 UPD. Also, children with mosaic genome-wide paternal UPD can present with a spectrum of additional phenotypic features that are typically associated with other imprinted disorders, e.g., significant developmental delay as seen in Angelman syndrome. Tumors have been a frequent feature in published cases of genome-wide paternal UPD and have included embryonal tumors associated with Beckwith-Wiedemann syndrome as well as rarer tumors with low metastatic potential such as hamartomas and pheochromocytomas. Data from population-based studies will be helpful in determining the true tumor risk in this condition. Given the clinical and molecular overlap with Beckwith-Wiedemann syndrome, most centers currently recommend the same screening protocol used for children with Beckwith-Wiedemann syndrome [52]. Molecular testing and diagnosis can be challenging for genome-wide UPD due to somatic mosaicism. Standard molecular testing for Beckwith-Wiedemann syndrome (MS-MLPA) may yield positive or negative test results. A high level of suspicion is required when encountering any child with features outside the usual Beckwith-Wiedemann syndrome spectrum, especially developmental delay [74–78]. In such cases, consultation with clinical and/or laboratory geneticists would be indicated to guide further testing and management.

7.6 Perlman Syndrome

Perlman syndrome is a rare overgrowth syndrome. It shares some features with Beckwith-Wiedemann syndrome [79, 80]. Prenatal findings may include polyhydramnios, macrosomia, nephromegaly, and ascites. A high neonatal mortality

(>50%) rate is reported due to pulmonary hypoplasia which may or may not be associated with renal insufficiency [79]. The typical facies are characterized by depressed nasal bridge, deep-set eyes, low-set dysplastic ears, and micrognathia. Developmental delay is a common feature of Perlman syndrome [79]. Histologically, the kidneys usually demonstrate nephroblastomatosis, which predisposes to the development of Wilms tumor. In Perlman syndrome, Wilms tumor can present as early as the neonatal period, and there is an exceptionally high incidence of bilateral Wilms tumor (>50%). Pancreatic islet cell hyperplasia is seen in 70% of cases [79]. Due to the shared features with Beckwith-Wiedemann syndrome, the methylation status of 11p15 has been investigated in patients with Perlman syndrome and found to be normal [80].

Recently, germline pathogenic variants in the gene *DIS3L2* on chromosome 2q37 have been linked to Perlman syndrome and Wilms tumor susceptibility [81]. This gene encodes a protein with ribonuclease activity suggesting that Perlman syndrome results from aberrant RNA metabolism. Patients with Perlman syndrome who survive the neonatal period are at high risk for Wilms tumor; hence, frequent abdominal exam and ultrasound surveillance are recommended [14].

Alterations in microRNA processing genes have frequently been described in sporadic Wilms tumors [67, 68]. In Perlman syndrome, *DIS3L2* variants play a role in *let-7* microRNA degradation and thus affect overall microRNA expression profiles [82]. Notably, one study showed that *DIS3L2* knockdown and knockout models in mice are useful for the study of Perlman syndrome and that they demonstrate constitutive IGF2 overexpression in the absence of epigenetic changes at chromosome 11p15.5 suggesting a common pathway with Beckwith-Wiedemann syndrome in Wilms tumor development [83].

7.7 Weaver Syndrome

Weaver syndrome is characterized by pre-and postnatal overgrowth, accelerated osseous maturation, characteristic craniofacial appearance, and intellectual disability [84]. Children with Weaver syndrome present with retrognathia, large fleshy ears, almond-shaped eyes, and variable degrees of intellectual impairment [2]. Some individuals have normal development/intellect. In terms of growth, children with Weaver syndrome are usually tall, but macrocephaly is a variable finding. Until recently, relatively few cases were diagnosed based on clinical findings. De novo pathogenic variants in *enhancer of zeste homolog 2* (*EZH2*) have been found in individuals with this syndrome [2, 85]. *EZH2* is the functional catalytic component of the polycomb repressive complex (PRC2). Variants in other components of the PRC2 complex- EED and SUZ12 – are associated with overgrowth syndromes that overlap clinically with Weaver syndrome. Recent work suggests that all Weaver-like syndromes with pathogenic variants in PRC2 components share a common pathogenic pathway reflected in a common pattern of genome-wide DNA methylation alterations or signature [86]. These signature DNA methylation changes can be used to aid in the diagnosis of Weaver syndrome especially in cases where genomic

testing reveals only variants of unknown significance. Similarly, a DNA methylation signature can be a useful adjunct in diagnosing patients with Sotos syndrome (see below) [87].

Both neuroblastoma and hematologic malignancies have been reported in children with Weaver syndrome [2, 88]. The ability to accurately diagnose children with Weaver syndrome using multi-modality molecular testing will facilitate better estimates of the risk of malignancy in this condition as it is possible that there is underascertainment of mild cases of Weaver syndrome in children whose first presentation is neuroblastoma. These studies will in the future clarify whether tumor surveillance should be undertaken for children with this diagnosis; however, at this time tumor surveillance is not considered the standard of care in this group.

7.8 Sotos Syndrome

The cardinal features of Sotos syndrome include a typical facial gestalt, learning disability, and overgrowth [2]. At birth, height is usually over two standard deviations for gestational age, but the weight is frequently normal. Children with Sotos syndrome often present with tall stature and macrocephaly. However, not all individuals with Sotos syndrome have tall stature or macrocephaly. Typical facial features include a high, broad forehead, sparse fronto-temporal hair, malar flushing, down-slanting palpebral fissures, and a prominent chin. Seizures, scoliosis, and cardiac and renal abnormalities have also been described [2]. Intellectual disability and behavioral problems are common [2].

From a molecular standpoint, pathogenic variants in the nuclear receptor SET domain-containing protein-1 (*NSD1*) gene on chromosome 5q35 cause Sotos syndrome. Inheritance follows an autosomal dominant pattern; with most cases arising from de novo mutations [89]. Studies of chromosome 11p15 in Sotos syndrome patients are typically normal.

Overall, the risk of tumor development in Sotos syndrome is approximately 3% [90]. Hematologic malignancies (leukemia, lymphoma) are more common in this patient population than embryonal abdominal tumors although Wilms tumor has also been reported [89, 91]. Interestingly, pathogenic variants in *NSD1* have been independently linked to childhood acute myeloid leukemia [1] as well as a variety of adult tumor types not associated with Sotos syndrome including glioma, breast cancer, and non-HPV-related nasopharyngeal carcinoma. Given the low penetrance of each tumor type, no specific screening protocol has been recommended for the broad range of tumors that can occur in Sotos syndrome.

7.9 Simpson-Golabi-Behmel Syndrome

Simpson-Golabi-Behmel syndrome is an X-linked condition with close to 100 patients reported in the literature [91, 92]. It is characterized variably by pre- and postnatal overgrowth, coarse facial features, macroglossia, intellectual disability, renal and skeletal abnormalities, and supernumerary nipples. Mutations or deletions causing loss of function of the protein encoded by the *GPC3* (glypican-3) gene on chromosome Xq26 have been described in association with the syndrome [93, 94]. *GPC3* encodes a cell surface proteoglycan that is involved in the regulation of cell division and growth [95]. Pathogenic variants in GPC3 are detected at rates of 37–70% in clinically diagnosed males. *GPC4* is contiguous with *GPC3* and is often included in diagnostic testing for Simpson-Golabi-Behmel syndrome. However, there are no clear cases of *GPC4* variants causing this syndrome in the absence of concomitant *GPC3* variants.

Embryonal tumors such as Wilms tumor and hepatoblastoma have been described in patients with Simpson-Golabi-Behmel syndrome. Wilms tumor appears to be the most common malignancy in this group with an estimated incidence of 3–5% [14, 96]. A case of hepatocellular carcinoma in a 3-year-old male with Simpson-Golabi-Behmel syndrome has been reported [92]. Also, there is a case report of an unusual tumor, ameloblastoma, in a child who had other histologically distinct cystic lesions in the mandible and maxilla although it is possible that the provenance of the oral tumors was separate from the diagnosis of Simpson-Golabi-Behmel syndrome in this case [97].

GPC3 is often found to be overexpressed at the cell surface in a variety of tumors, most prominently hepatocellular carcinoma but also hepatoblastoma and germ cell tumors [98–100]. Although one study on mechanisms of GPC3-related oncogenesis in hepatocellular carcinoma demonstrated the involvement of the *IGF2* signaling pathway [101] similar to Beckwith-Wiedemann syndrome, other evidence suggest that its role in oncogenesis is more related to the Wnt signaling pathway, an important driver of various tumor types. Despite its prominence in several tumor types, the relationship between *GPC3* and cancer, where overexpression is commonly involved, is different from its role in Simpson-Golabi-Behmel syndrome where loss-of-function variants predominate.

Tumor screening protocols recommended for Simpson-Golabi-Behmel syndrome typically parallel the surveillance practiced for Beckwith-Wiedemann syndrome [14]. As this is an X-linked condition, the risk to have a child with Simpson-Golabi-Behmel syndrome for carrier females is significant. Therefore, genetic counseling including genetic testing for at-risk females and for individuals with this condition is highly recommended.

7.10 Segmental Overgrowth Syndromes Caused by Variants in the PI3K-AKT Pathway

Some segmental overgrowth syndromes have been shown to be caused by pathogenic variants in genes in the PI3K-AKT pathway (Proteus syndrome, *PIK3CA*-related overgrowth syndromes). While these children have more tumors than the general population, many of the tumors are benign, and a detailed review of management is beyond the scope of this chapter. We recommend referral to a clinical geneticist for any child suspected of having one of these syndromes as diagnosis can be difficult. Tumor surveillance for each child should be discussed taking into account factors including that child's particular risk for tumor development, the likelihood of early tumor detection, and patient/parental preferences [102–105].

7.11 Conclusion

Defining accurate molecular and clinical diagnoses for children with overgrowth syndromes can facilitate appropriate management strategies as well as counseling for them and for their families. In particular, the ability to molecularly classify such children will support prospective studies to accurately define tumor risk and the spectrum of tumor types for each condition or molecular subgroup. A persisting challenge is the fact that somatic mosaicism can complicate both the ability to diagnose and the assignment of relevant tumor risks. The spectrum of phenotypes in these syndromes behooves the oncology clinician to consider overgrowth syndromes when treating any child with related tumors, especially Wilms tumors and hepatoblastoma. We expect that novel genomic testing strategies will soon support the development of personalized risk assessment and surveillance strategies for children with a variety of overgrowth syndromes associated with embryonal tumor predisposition.

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Chapter 8
Multiple Endocrine Neoplasias and Associated Non-endocrine Conditions

Rebekah K. Jobling and Jonathan D. Wasserman

Abstract Tumors of hormone-producing tissues are frequent among patients with hereditary cancer syndromes. Lesions in multiple tumor suppressor genes and oncogenes are responsible for several distinct hereditary tumor syndromes with autosomal dominant inheritance. These include multiple endocrine neoplasia (MEN) types 1, 2, and 4, Carney complex, Cowden syndrome, hereditary pheochromocytoma/paraganglioma syndromes, and von Hippel-Lindau disease in addition to other diagnoses including neurofibromatosis type 1 and tuberous sclerosis that frequently have endocrine manifestations. Signs and symptoms of hormone excess are the frequent initial finding among patients with endocrine tumors, although mass effect may be the presenting complaint, particularly among non-functional tumors. More recently, presymptomatic screening of at-risk patients has allowed for earlier detection and intervention, with a resultant decrease in mortality and morbidity associated with these tumors.

Keywords Multiple endocrine neoplasia · Parathyroid · Medullary thyroid carincoma · Pituitary adenoma · Pancreatic neuroendocrine tumor · MEN1 · MEN2 · RET gene

R. K. Jobling
Division of Clinical and Metabolic Genetics, Department of Paediatrics, Hospital for Sick Children, University Avenue, Toronto, Ontario, Canada

Department of Paediatrics, University of Toronto, Toronto, ON, Canada

J. D. Wasserman (✉)
Department of Paediatrics, University of Toronto, Toronto, ON, Canada

Division of Endocrinology, Department of Paediatrics, Hospital for Sick Children, University Avenue, Toronto, Ontario, Canada
e-mail: Jonathan.wasserman@sickkids.ca

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8.1 Introduction

Tumors of hormone-producing tissues are frequent among patients with hereditary cancer syndromes. Lesions in multiple tumor suppressor genes and oncogenes are responsible for several distinct hereditary tumor syndromes with autosomal dominant inheritance. These include multiple endocrine neoplasia (MEN) types 1, 2, and 4, Carney complex, Cowden syndrome, hereditary pheochromocytoma/paraganglioma syndromes, and von Hippel-Lindau disease in addition to other diagnoses including neurofibromatosis type 1 and tuberous sclerosis that frequently have endocrine manifestations.

Signs and symptoms of hormone excess are the frequent initial finding among patients with endocrine tumors, although mass effect may be the presenting complaint, particularly among non-functional tumors. More recently, pre-symptomatic screening of at-risk patients has allowed for earlier detection and intervention, with a resultant decrease in mortality and morbidity associated with these tumors.

This chapter will address the clinical and molecular associations of MEN syndromes including diagnosis, screening, surveillance, and ethical issues inherent to the management of affected patients and families.

8.2 History

Accounts of post-mortem findings of patients with multiple endocrine tumors can be found as far back as 1903, when Erdheim described the case of an acromegalic patient with poly-glandular parathyroid adenomas and pituitary adenoma in an individual with what was most likely MEN 1 [1]. Exactly 50 years later, Underdahl reported a series of eight patients with pituitary, pancreatic, and parathyroid tumors [2]. Subsequently, in 1954, Wermer recognized the dominant transmission of a syndrome with pituitary and parathyroid adenomas and pancreatic islet cell tumor [3].

The entity now recognized as MEN 2A was initially described in 1961 by Sipple, who identified the combination of parathyroid adenoma, pheochromocytoma, and medullary carcinoma of the thyroid [4], although Sisson et al. retrospectively identified what is probably the first description of MEN 2A-related parathyroid disease, from 1939, in a retrospective analysis of pathological specimens [5]. An excellent narrative history of the identification and subsequent classification of the multiple endocrine neoplasia syndromes was recently published [6].

Progress over the past several years has led to the identification of the genetic underpinnings of these conditions and has established guidelines for diagnosis, treatment, and surveillance.

The multiple endocrine neoplasias present with a spectrum of endocrine and non-endocrine manifestations. Extensive work over the past decades has identified exquisite detail regarding their molecular etiology, and diagnostic and management guidelines have emerged based on this understanding. Clinical manifestations of

Table 8.1 Distribution and frequencies of major phenotypes of MEN 1, 2A, and 2B

MEN 1	MEN 2A	MEN 2B
Pituitary adenoma (30–55%)	Medullary thyroid carcinoma (>90%)	Medullary thyroid carcinoma (100%)
Parathyroid hyperplasia (95%)	Parathyroid hyperplasia (0–20%)	Marfanoid habitus, alacrima, mucosal neuromas, etc.
Gastro-entero-pancreatic neuroendocrine tumors (50–75%)	Pheochromocytoma (0–50%)	Pheochromocytoma (~50%)

MEN 1, 2A, and 2B are summarized in Table 8.1, whereas the full clinical spectrum of the more recently identified MEN 4 remains to be clearly articulated.

8.3 Multiple Endocrine Neoplasia 1 and Isolated Primary Hyperparathyroidism

Multiple endocrine neoplasia 1 (MEN 1) comprises hyperplasia or neoplasia of the parathyroid glands, gastro-entero-pancreatic neuroendocrine cells, and anterior pituitary gland. It results from germline variants in the *MEN1* gene in 80–90% of cases [7–10]. De novo variants account for ~10% of MEN 1 cases.

8.3.1 Diagnosis of MEN 1

Diagnosis of MEN 1 is established by the presence of two or more of the major MEN 1-constituent tumors including:

1. *Parathyroid hyperplasia*: Manifest as hypercalcemia with un-suppressed PTH. MEN 1 patients with hypercalcemia due to parathyroid hyperplasia are frequently asymptomatic, although some may exhibit symptoms of hypercalcemia including:
   (a) **CNS Disturbances** – Fatigue, altered mental status (lethargy, depression, confusion).
   (b) **Gastrointestinal Disturbances** – Anorexia, constipation, nausea, and vomiting.
   (c) **Renal** – Polyuria, impaired concentrating ability, dehydration, and nephrolithiasis.
   (d) **Skeletal** – Bone pain and increased fracture risk.
   (e) **Cardiovascular** – QT interval shortening, hypertension.
2. *Pituitary Tumors*: Predominantly prolactinoma which often presents with headache and/or bitemporal hemianopsia. In females, hyperprolactinemia associated

with prolactinoma presents with oligo-amenorrhea and galactorrhea and in males with sexual dysfunction and (rarely) galactorrhea. Growth hormone-secreting tumors are associated with excess growth. ACTH-secreting tumors result in Cushing's syndrome.

3. *Gastro-entero-pancreatic Neuroendocrine Tumors (GEP-NETs)* [11]: Manifestations are dependent on the cell of origin and include (in order of most to least frequent):
   (a) Non-secretory Pancreatic NETs: Identified due to mass effect or based on pre-symptomatic imaging.
   (b) Gastrinoma: Zollinger-Ellison syndrome (ZES)→peptic ulcer ± chronic diarrhea.
   (c) Insulinoma: Recurrent hypoglycemia, weight gain.
   (d) Glucagonoma: Hyperglycemia, anorexia, glossitis, anemia, diarrhea, venous thrombosis, and skin rash.
   (e) VIPoma: Watery diarrhea, hypokalemia, and achlorhydria.

8.3.2 *Prevalence*

The estimated prevalence of MEN 1 ranges between 1:5000 and 1:50,000 in Caucasian populations [12, 13].

8.3.3 Clinical Presentation

Multiple endocrine neoplasia 1 (MEN 1) is a condition with high penetrance, high morbidity, and substantial cause-specific mortality. Timely diagnosis and intervention are thus necessary to mitigate the inevitable manifestations among disease carriers.

While MEN 1 may present with any of the constituent tumors, the likelihood and timing of developing individual components of MEN 1 vary as summarized in Table 8.2. Primary hyperparathyroidism is the most common presenting feature of MEN 1 [15, 16] and occurs in approximately 95% of MEN 1 patients [17, 18]. Pancreatic neuroendocrine tumors (consisting of gastrinomas, insulinomas, glucagonomas, VIPomas, and non-secretory tumors) occur in 40–75% of MEN 1 patients [17], whereas pituitary adenomas (predominantly prolactinomas and, less commonly, growth hormone (GH)-secreting adenomas) are identified in 30–55% [19, 20]. In children, Cushing's disease (ACTH-secreting pituitary adenoma) may be the initial manifestation [21]. Age of onset is similarly variable and drives recommendations for screening in affected individuals (see Table 8.4).

Table 8.2 Tumors associated with MEN 1 and estimated disease penetrance. Used with permission from [14]

Tumor	Penetrance, %
Parathyroid tumors	**90**
GEP-NETs	**30–70**
Non-functioning	20–55
Gastrinoma	40
Insulinoma	10
Glucagonoma	<1
VIPoma	<1
Pituitary adenomas	**30–40**
Prolactinoma	20
Somatotropinoma	10
Corticotropinoma	5
Non-functioning	5
Associated tumors	
Adrenocortical tumor	40
Pheochromocytoma	<1
Bronchopulmonary neuroendocrine tumor	2
Thymic neuroendocrine tumor	2
Gastric neuroendocrine tumor	10
Lipoma	30
Angiofibroma	85
Collagenoma	70
Meningioma	8

Patients with MEN 1 are at increased risk of premature death [22, 23] with malignant pancreatic neuroendocrine tumors the leading cause of death [24, 25]. It has been estimated that 1/3 of patients with MEN 1 will die early from an MEN-related cancer. In one Dutch series, the average age of death was 55.4 years for men and 46.8 for women [23]. There has been a slight decrease in mortality over the past 20 years [25], presumably due to earlier detection of intra-abdominal tumors, and this has prompted calls for more aggressive abdominal imaging among *MEN1* variant carriers to further mitigate the risks of death from metastatic disease.

8.3.3.1 Primary Hyperparathyroidism (PHPT)

Hyperparathyroidism occurs in up to 95% of patients with MEN 1 and is the presenting manifestation in most cases. In patients with MEN 1, multi-gland hyperplasia predominates, in contrast to sporadic PHPT, which results from solitary adenomas in 89% of cases [26]; thus, identification of patients with poly-glandular parathyroid hyperplasia should prompt careful review for other components of MEN 1 or, less commonly, MEN 2A. PHPT presents at younger ages in MEN 1 (mean age = 19 years) than in sporadic PHPT (mean age = 50s) [27].

Hyperparathyroidism may present as asymptomatic hypercalcemia in the setting of un-suppressed parathyroid hormone (PTH) or may present symptomatically with signs and symptoms typical of hypercalcemia including renal calculi, abdominal pain, constipation, behavioral changes, bony pain, brown tumors of bone, etc. Hyperparathyroidism tends to be more severe in patients with MEN 1 than those with MEN 2A.

Familial isolated primary hyperparathyroidism (in the absence of other MEN features) has also been described and is frequently associated with germline *MEN1* variants.

Parathyroid carcinoma is not known to be associated with MEN 1, although it has been described among patients with the familial hyperparathyroidism-jaw tumor syndrome (HPT-JT) which results from variants at the *HRPT2* locus [28].

8.3.3.2 Gastro-Entero-Pancreatic Neuroendocrine Tumors (GEP-NETs)

GEP-NETs comprise both pancreatic NETs (islet cell and non-islet neoplasms) and NETs of the alimentary tract, thymus, and bronchi (formerly termed "carcinoids"). Neuroendocrine tumor (NET) is the current accepted terminology for a neoplasm of the neuroendocrine tissues, following a WHO consensus conference in 2000 [29]. The mean age of diagnosis in one series of patients under surveillance was 41.4 years, with 20.8% of patients diagnosed with GEP-NET prior to 30 years [30]. Although classically neuroendocrine tumors were identified based on their secretory products, non-functioning tumors have been shown to be the most common GEP-NET and are associated with worse prognosis than functional NETs in MEN 1 [31, 32]. Pancreatic NETs occur in 30–80% of patients with MEN 1 and are the most common cause of death. They are estimated to have undergone metastasis to regional lymph nodes at the time of diagnosis in ~50% of patients. Similarly thymic NETs are associated with increased risk of death [25], while bronchial NETs tend to remain indolent and are not associated with increased mortality [33].

Gastrinomas develop in up to 54% of *MEN1* variant carriers and are located in the duodenum 90% of the time [12]. They may also be found in the head of the pancreas. They typically present with the Zollinger-Ellison syndrome (ZES) with fasting serum gastrin >1000 pg/mL and gastric pH < 3. Patients may present with esophagitis, peptic ulcer disease, and/or secretory diarrhea.

Secretory pancreatic islet tumors include insulinomas, VIPomas, glucagonomas, and pancreatic polypeptide-omas. Insulinomas are the most common secretory tumor in patients <25 years, although they remain rare, occurring in <10% of patients with MEN 1. Only 10% of patients with insulinomas have MEN 1 [27]. Insulinomas are associated with Whipple's triad (documented hypoglycemia, associated neuroglycopenic symptoms, and resolution of symptoms with correction of hypoglycemia) and weight gain. Hyperinsulinemic hypoglycemia is formally diagnosed in the setting of a 72-h fast.

8.3.3.3 Pituitary Adenomas

Anterior pituitary adenomas are identified in 30–55% of patients with MEN 1 and are most frequently prolactinomas, although growth hormone-secreting tumors (~25% of MEN 1-associated pituitary tumors) and ACTH-secreting tumors (~5%) have also been described. Roughly 1–3% of pituitary adenomas are identified in patients with MEN 1, although this figure rises to 14% when considering only prolactinomas [27]. Age of onset of pituitary adenoma in MEN 1 ranged from 12 to 83 years (with most diagnosed before age 50), in one series [20], although the youngest patient reported with MEN 1-associated pituitary adenoma was 5 years old [34].

8.3.3.4 Adrenocortical Adenomas

Adrenocortical adenomas are identified in 35% of individuals with MEN 1. They are most frequently non-secreting, but may produce glucocorticoids or mineralocorticoids, presenting with Cushing's or Conn's syndrome, respectively. They are typically benign with only rare reports of malignant transformation among patients undergoing radiologic surveillance [35]. Adrenal medullary tumors (pheochromocytomas) are rare in MEN 1.

8.3.3.5 Other Manifestations of MEN 1

Beyond the core tumor types in MEN 1, as many as 20 endocrine and non-endocrine neoplasias are associated with the syndrome, including:

- Lipomas [36].
- Leiomyomas [37].
- CNS tumors.
  - Ependymoma [38, 39].
  - Meningioma [40].
- Cutaneous tumors [41, 42].
  - Collagenomas.
  - Facial angiofibromas.

8.3.4 MEN 1 Phenocopies

Despite a diagnosis of familial MEN 1, it has been estimated that 5–10% of MEN kindreds include phenocopies (disease manifestations associated with the syndrome in *MEN1*^{wt} individuals, attributable to other causes) [43–45]. MEN phenocopies are

common including pituitary and parathyroid disease, likely due to the common occurrence of these diagnoses in the general population; thus, genetic testing is indicated to confirm a clinical diagnosis of MEN 1.

8.3.5 Management

Manifestations of MEN are multifocal, multiglandular, and often recurrent; thus, determination of indications for and timing of surgical interventions is complex. A detailed description of treatment of the many manifestations of MEN 1 is beyond the scope of this review. Clinical practice guidelines have been developed and are publicly available [16, 18, 46, 47]. Referral to an experienced endocrinologist and surgeon familiar with the care of these patients is strongly encouraged. Issues surrounding surgical interventions among *MEN1* variant carriers have been reviewed [14, 46, 48, 49].

Hyperparathyroidism is treated by subtotal parathyroidectomy with cryopreservation of parathyroid tissue or by total parathyroidectomy and auto-transplant of parathyroid tissue (generally into the non-dominant brachioradialis) [50]. Indications for surgical intervention for MEN 1-related hyperparathyroidism align with those for sporadic hyperparathyroidism. Surgery is generally warranted for any patient with hyperparathyroidism age < 50 years (regardless of symptoms) or those with:

- Osteoporosis, fragility fractures, or vertebral compression fractures; or
- Serum calcium >1 mg/dL (0.25 mmol/L) above the reference range; or
- Renal involvement: nephrocalcinosis, nephrolithiasis, hypercalciuria, or glomerular filtration rate < 60 mL/min.

Early surgical intervention has been advocated, as individuals with MEN 1 may experience decreased bone mineral density and/or diminished renal function even if asymptomatic or only mildly hypercalcemic [51, 52].

Pituitary adenomas—The goals of treatment are reduction in tumor volume (and associated mass effect) and decrease in hormone hypersecretion. Treatment can include medical therapy, surgery, or radiation therapy.

Prolactinomas are treated with dopamine agonists (cabergoline is the preferred choice, although bromocriptine has also been employed). Surgical options are to be considered only in circumstances of impending vision loss or hemorrhage, as medical therapy is generally effective in reducing tumor volume and hormone hypersecretion.

Growth hormone-secreting adenomas are treated with transsphenoidal resection, although somatostatin analogs (octreotide and lanreotide) may be an option for poor surgical candidates.

Other pituitary tumors are most often resected if symptomatic.

GEP-NETs are resected once localized. Gastric acid hypersecretion may be managed initially with proton-pump inhibitors and/or H_2 receptor blockers; however, gastrinomas are associated with high rates of malignancy and nodal metastases [53],

and thus careful staging and surgical exploration are advised. An algorithm for determining operative approach is summarized by Dickson and colleagues [53].

Surgery for non-functional pancreatic NETs is indicated for tumors >2 cm or < 2 cm with rapid growth [54, 55, 56], although there is some controversy regarding management of tumors 2–3 cm [57].

Adrenocortical tumors, although rarely malignant in MEN 1, are generally resected when they exceed 3.0 cm in diameter.

8.3.6 Molecular Genetics of MEN 1

8.3.6.1 Gene

MEN 1 results from variants in the *MEN1* gene, located on chromosome 11q13. The *MEN1* gene is spread across ten exons. *MEN1* encodes Menin, a 610-amino acid protein with multiple suspected functions including cell cycle control, maintenance of genomic stability, and transcriptional regulation (reviewed in [10, 58]).

Menin is a novel protein, ubiquitously expressed with no identifiable homology to other proteins. It is a tumor suppressor, thought to be a nuclear protein based on in vitro studies, and appears to mediate interactions with other nuclear proteins, including AP1 and cJun, and inhibits transcriptional activation by JunD [59–61]. In addition, it appears to be a component of an MLL/SET1 histone methyltransferase (HMT) complex that methylates lysine 4 of histone 3 (H3K4), thereby regulating transcription. Additionally it appears to bind the *hTERT* promoter and repress telomerase expression.

Tumor formation in MEN 1 is thought to arise according to the two-hit hypothesis of Knudson, whereby a single mutant allele is present in the germline, while a second somatic variant or loss of the wild-type allele is necessary for tumorigenesis. Loss of heterozygosity (LOH) is frequently encountered in tumors arising in *MEN1* variant carriers [62, 63].

MEN1 is also recognized to be a major driver of somatic parathyroid neoplasia. Whole-exome analysis of sporadic parathyroid adenomas identified somatic variants in *MEN1* in 35% of tumors among individuals without other manifestations of MEN 1 [64].

The penetrance of *MEN1* variants is estimated at 45%, 82%, and 96% at 30, 50, and 70 years of age, respectively, for diagnosis of a first feature of MEN 1 in variant carriers [16].

8.3.6.2 Genetic Testing

Indications for testing are several-fold. Primarily, confirmation of *MEN1* variants among individuals with suspected MEN 1 can validate clinical diagnosis. Second, mutational confirmation can help direct pre-symptomatic screening in an

age-appropriate manner for variant-positive individuals. Finally identification of a familial variant facilitates genetic screening of relatives, to identify those unaffected individuals who may be spared the burden, cost, and psychological impact of cancer predisposition.

Current guidelines advocate genetic testing when one any of the following criteria are met:

1. Any individual with two or more core MEN 1 tumors (primary hyperparathyroidism, anterior pituitary adenoma, or pancreatic neuroendocrine tumor).
2. First-degree relatives of an individual with known *MEN1* variant with signs, symptoms, and biochemical or radiographic evidence of any MEN 1 component tumors.
3. Asymptomatic first-degree relatives of individuals with known *MEN1* variants.
4. Suspicion for "atypical" MEN 1 such as [65]:
   (a) Parathyroid adenoma diagnosed prior to age 30.
   (b) Multiglandular or recurrent parathyroid adenoma.
   (c) Gastrinoma at any age.
   (d) Multiple pancreatic NETs at any age.
   (e) One "core" MEN 1 tumor (see #1) PLUS any associated manifestations (such as adrenocortical tumors, lipomas, angiofibromas, collagenomas, or extra-pancreatic NETs (bronchial, duodenal, thymic, etc.))

de Laat and colleagues derived a nomogram for identification of individuals likely to carry *MEN1* variants [66] (Fig. 8.1).

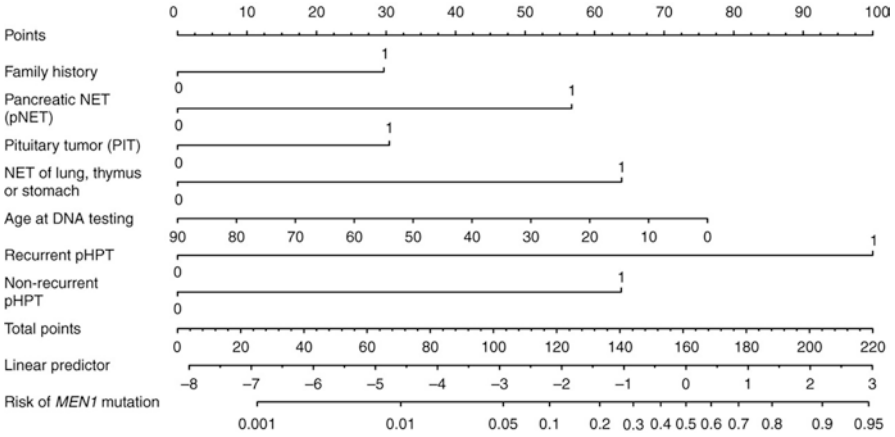

Fig. 8.1 Nomogram for predicting likelihood of *MEN1* variant. *NET* neuroendocrine tumor, *pHPT* primary hyperparathyroidism. Example: a 54-year-old patient (score = 30 points) with the combination of a negative family history (score = 0 points), a non-recurrent and non-multiglandular pHPT (score = 63 points), and a pNET ($n = 57$ points) has a sum score of 150 points corresponding with a linear predictor of −0.50 and a risk of 38% of having a *MEN1* variant. Reproduced with permission from [66]

For individuals meeting any of these criteria, genetic testing should be undertaken as early as possible, since tumors have developed in children as young as 5 years old. Nevertheless, there is an age-related penetrance of MEN 1 manifestations, with only 50% of patients younger than 20 years having any manifestations, while >99% of variant-positive patients over 45 with at least one manifestation [18, 67].

Confirmation of a diagnosis in a proband should include sequence analysis of *MEN1* first, and if no variant is found, deletion/duplication analysis should follow. Whole genome sequencing identified *MEN1* variants in 3/14 individuals with a clinical diagnosis of MEN 1, but previously normal genetic testing [68]. Predictive testing for at-risk asymptomatic individuals, prenatal diagnosis, and pre-implantation diagnosis require prior identification of a familial variant.

In simplex cases (wherein only a single family member is affected) that meet diagnostic criteria, up to 65% of individuals will carry a germline *MEN1* variant [69]. When two or more family members are affected, this value increases to 80–95% [17, 70].

Familial isolated hyperparathyroidism (FIHP) is, by definition, not associated with other endocrine neoplasia. Between 20% and 57% of families with FIHP carry germline variants in *MEN1* [71–73]. As with MEN 1, there is no clear genotype-phenotype predictor of FIHP, although with FIHP, missense variants are found more frequently and nonsense variants are rarer [10].

8.3.6.3 Genotype/Phenotype Relationships

Variants in *MEN1* are scattered across the gene, with no defined mutational hotspots (Fig. 8.2) [10, 75, 76]. More than 1300 variants have been identified to date [7–10]. The vast majority of variants are nonsense or frameshift variants that result in truncated proteins; the remainder are missense or intragenic deletions that lead to the expression of an altered protein. There is no clear genotype-phenotype correlation, and substantial variability in disease presentation (including age of onset, severity of disease, and tumor types) within and between pedigrees is common. It has been estimated that more than 10% of germline *MEN1* variants occur de novo. Moreover up to 10% of individuals with clinically diagnosed MEN 1 have no detectable

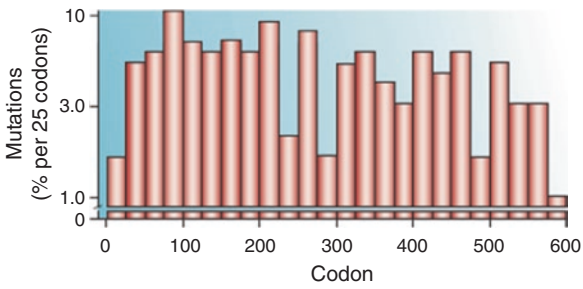

Fig. 8.2 Distribution of variants in MEN 1 by codon. There are no apparent mutational hotspots. Reproduced with permission from [74]

Table 8.3 Prevalence of *MEN1* variants among common presenting MEN 1 conditions and alternative genetic etiologies

Diagnosis	Proportion of patients with diagnosis with MEN1 variant	Reference	Alternative syndromic diagnosis (gene)
Zollinger-Ellison syndrome	~33%	[77, 78]	
Hypercalcemia/ hyperparathyroidism	6–20%	[16, 79, 80]	• HPT-jaw tumor syndrome (*HPRT2*) • Familial hypocalciuric hypercalcemia (*CaSR*)
Pituitary adenoma	<1–5%	[81–83]	• Familial isolated pituitary adenoma (*AIP*)
GEP-NETs Insulinoma Thymic NET	10% 25%	[16] [84, 85]	• Tuberous sclerosis (*TSC1/2*), • Neurofibromatosis (*NF1*)-insulinoma, somatostatin/ serotonin-secreting NETs • von Hippel-Lindau (*VHL*)

exonic or splice-site variants in *MEN1* [10]. The prevalence of manifestations of MEN 1 is summarized in Table 8.3.

8.3.7 Pre-Symptomatic Screening and Surveillance

Age-specific recommendations for clinical and biochemical surveillance of known *MEN1* variant carriers have been defined, based on the youngest age at which manifestations of disease were reported [16–18]. At our institution, we have implemented earlier abdominal imaging, in the setting of reports of non-secretory pancreatic NETs in patients as young as 12 years old [86]. Our screening regimen, based largely on those defined previously [17, 18], is detailed in Table 8.4.

For first-degree relatives of *MEN1* carriers with *unknown* variant status, the following surveillance regimen is suggested [17, 18]:

From 5 years:

- Annual serum prolactin.
- Assessment of growth velocity.

From 10 years:

- Annual fasting serum total calcium (corrected for albumin) and/or ionized calcium.
- Annual fasting intact PTH.

From 20 years:

- Fasting serum gastrin if symptomatic for ZES (reflux/esophagitis/diarrhea).

Table 8.4 Pre-symptomatic screening regimen for carriers of MEN1 variants as used at the authors' institution. Reproduced with permission from [47]

MEN1 manifestation	Screen starting at age	Clinical screening	Annual biochemical tests	Imaging
Insulinoma	5 yrs	Syncope, light-headedness, documented hypoglycemia	Fasting glucose and insulin	None
PitNET	5 yrs[a]	Headaches, visual changes, galactorrhea, ⇑ growth	Prolactin, IGF-1	Brain MRI (q 3 years)
Parathyroid adenoma/1° HyperPTH	8 yrs	Back pain, bone pain, weakness, fatigue, psychiatric changes, kidney stones, nausea, vomiting, constipation. Multiple or pathologic fractures	Calcium[b]	None
Pancreatic NET	10 yrs	Generally not identified symptomatically. VIPoma can cause profuse diarrhea. Glucagonoma associated with hyperglycemia, nausea, polyuria, thirst	(Chromogranin A, glucagon, proinsulin, pancreatic polypeptide, VIP)[c]	Abdominal MRI (annually)
Adrenal adenoma	10 yrs	None	None	Abdominal MRI (contemporaneous with pancreatic imaging)
Gastrointestinal, bronchial, and thymic NETs	20 yrs	Frequently asymptomatic, but h/o flushing, diarrhea, wheezing, edema, or abdominal pain should arouse suspicion		CT/MRI chest and abdomen (q 1–2 years)
Gastrinoma (duodenal and pancreatic)	20 yrs	Abdominal pain, gastric ulcers. Proton-pump inhibitor usage	Fasting gastrin	None

Data from Thakker et al.; ref. 5

h/o history of, *HyperPTH* hyperparathyroidism, *NET* neuroendocrine tumor, *q* every, VIP vasoactive intestinal polypeptide, *yrs* years

[a]MRI surveillance is to begin once patient is able to tolerate a nonsedated MRI. In the authors' experience, this is generally at about the age of 5 years but may be deferred on an individualized basis

[b]Hypercalcemia on screening should prompt assessment with contemporaneous serum calcium and intact parathyroid hormone (iPTH) to establish a diagnosis of PHPT

[c]Pancreatic tumors may be nonsecretory; therefore, the added sensitivity contributed by biochemical screening has not been demonstrated

8.4 Multiple Endocrine Neoplasia Type 2 and Familial Medullary Thyroid Cancer

8.4.1 Diagnosis of MEN2

Multiple endocrine neoplasia type 2 (MEN 2) constitutes three distinct syndromes, all arising from variants in the *RET* proto-oncogene. MEN 2A is characterized by the presence of medullary thyroid cancer (MTC) and variable presence of pheochromocytoma (PHEO) and primary hyperparathyroidism (PHPT). The MEN 2B phenotype also comprises MTC and PHEO, but to the exclusion of PHPT, and is further characterized by distinctive features such as widespread neurogangliomas and typical skeletal and facial appearance. FMTC is defined as the presence of medullary thyroid cancer in four or more family members of varying ages without other features of the MEN 2 syndromes. Based on mutational segregation, FMTC is now thought to represent a subtype of MEN 2A [87]. The MEN 2B phenotype accounts for 5–10% of all cases of MEN 2 and is the most aggressive subtype with respect to MTC [88]. De novo variants account for 5% of MEN 2A cases and up to 50% of MEN 2B [89].

8.4.2 Prevalence

Prevalence of MEN 2 has been estimated at 1:35000–40,000 [90, 91]. MEN 2A phenotype accounts for 56% of these patients, MEN 2B 9%, and FMTC the remaining 35%.

8.4.3 Clinical Presentation

Medullary thyroid carcinoma (MTC) is the clinical manifestation that unites all phenotypes associated with *RET* variants. It is most commonly diagnosed in childhood for those with MEN 2B, in early adulthood in MEN 2A, and in middle age for those with isolated FMTC. The average age of MTC onset in MEN 2B is approximately 10 years earlier than MEN 2A [92]. MTC is a tumor of parafollicular C-cells of the thyroid. Development of MTC in MEN 2 patients is preceded by C-cell hyperplasia. While MTC typically presents as a painless neck mass ± cervical adenopathy, it may also be associated with the following:

- Bilateral and multifocal onset of disease.
- Lymphatic spread to cervical and mediastinal lymph nodes and distant metastases in the bone marrow, liver, lungs, and bone [93].
- Hypersecretion of calcitonin and an elevated basal serum calcitonin level (>40 pg/mL) [94], although calcitonin levels alone should not be relied on as an indication for surgery, as the false-positive rate is estimated at ~ 6.6% [95].

- In patients with advanced disease, diarrhea or flushing episodes due to elevated calcitonin or calcitonin-like peptide [87].

Survival of MTC is related to the extent of metastasis at diagnosis. Overall 10-year survival is estimated at between 61% and 76% [96, 97]. Among patients with familial MTC where disease is detected based on screening, diagnosis is made at younger age and lower stage, and there is no excess mortality versus the general population, thus reinforcing the merit of cascade genetic testing and pre-symptomatic screening for at-risk individuals [96, 98, 99].

Pheochromocytoma (PHEO), like MTC, is associated with both MEN 2A and 2B. Lifetime risk for PHEO in patients with either MEN 2A or 2B is approximately 50% [100]. Penetrance of PHEO and age of diagnosis is highly correlated with MTC, based on *RET variant* [101]. Patients with MEN 2 are unlikely to develop PHEO until adulthood although cases have been described in patients as young as 8 years of age. Age at which to commence pre-symptomatic screening is based on the specific *RET* variants identified (see "Pre-symptomatic Screening and Surveillance"). Familial PHEO in the absence of relatives with MTC may be reflective of lesions at other genetic loci including *VHL, NF1, SDHx (A-D), TMEM127,* or *MAX* [102–107] .

- In children, symptoms most commonly include sustained hypertension (93%), headache (95%), sweating (90%), and visual disturbances (80%) [102].
- Malignant transformation of PHEO in MEN 2 is extremely rare in all age groups [108].
- Pheochromocytoma is a rare cause of hypertension in children and has been estimated to be the underlying diagnosis in approximately 1% of hypertensive pediatric patients [109, 110].

Primary hyperparathyroidism (PHPT) is observed only in MEN 2A. It is usually diagnosed in adulthood, but has been found in patients as young as 5 years [111, 112]. Primary hyperparathyroidism causes hypercalcemia, the symptoms of which are described above. PHPT is generally milder in MEN 2A than it is in MEN 1 and develops in 20–30% of individuals with MEN 2A [113]**.**

8.4.4 Non-endocrine Manifestations of MEN2

8.4.4.1 Non-endocrine Manifestations of MEN 2A

- Cutaneous lichen amyloidosis, a disorder of intense episodic pruritus, usually in the interscapular area, which is accompanied by skin changes such as hyperpigmentation, primarily among carriers of *RET* variants in codons 634 or 804.
- Some *RET* loss-of-function variants are associated with Hirschsprung disease (HSCR) in the context of MEN 2A/FMTC [114, 115]. Variants in *RET* are responsible for 50% of familial cases of Hirschsprung and 15–20% of sporadic cases [116].

8.4.4.2 Non-endocrine Manifestations of MEN 2B

MEN 2B is associated with a number of phenotypic features which may aid in recognition and diagnosis (Fig. 8.3):

(a) **Mucosal ganglioneuromas** at a variety of sites, including the tongue, lips, conjunctiva, and urinary system, and throughout the intestinal tract. Ganglioneuromas in the intestinal tract can lead to symptoms of constipation, vomiting, dehydration, and megacolon. In infants, they can manifest as feeding problems (Fig. 8.4b,d).
(b) **Diffuse intestinal ganglioneuromatosis** is present in approximately 40% of patients with MEN 2B [117], although symptoms of intestinal disturbance (including constipation, abdominal distention, and discomfort) affect up to 90% [118] (Fig. 8.4c).
(c) **Skeletal findings** including marfanoid body habitus, narrow long facies, pes cavus, pectus excavatum, high-arched palate, scoliosis, slipped capital femoral epiphysis, and joint laxity (Fig. 8.4a).
(d) **Other findings:** Hypotonia, proximal muscle weakness, eye findings (inability to make tears, thickened and everted eyelids, and prominent corneal nerves, Fig. 8.4d,e), and delayed puberty [118, 119].

8.4.5 Management

8.4.5.1 Medullary Thyroid Carcinoma

Surgery is the primary treatment modality for MTC and is the only modality that can achieve prevention or cure. Once metastatic, medullary thyroid cancer is associated with low cure rate and high morbidity and mortality. Early detection and treatment is thus crucial to avoiding complications [121]. As a result, prophylactic total thyroidectomy is the treatment of choice and is associated with a high cure rate if completed early in the disease progression [122]. The timing of prophylactic thyroidectomy for different risk levels is summarized in Fig. 8.4. The American Thyroid Association strongly recommends surgery at a high-volume center, due to the difficulty of the surgery in infants and young children [123]. In some cases, cure can be achieved by careful lymph node dissection after total thyroidectomy has failed to achieve a normalization of calcitonin or in the context of affected cervical lymph nodes with no other metastatic disease. Metastatic MTC, however, is not responsive to cytotoxic chemotherapy [124]. Kinase inhibitors cabozantinib and vandetanib have been approved and can extend progression-free survival, but not overall survival [125–127], and are associated with significant dose-limiting toxicities [128]. More recently, inhibitors of the *RET* receptor tyrosine kinase, selpercatinib (LOXO-292) and pralsetinib (BLU-667), have demonstrated efficacy and low toxicity in the treatment of *RET*-driven tumors [129].

Importantly, PHEO must be excluded in MEN 2 patients of any age prior to surgery, although of PHEO prior to age 11 is rare [98].

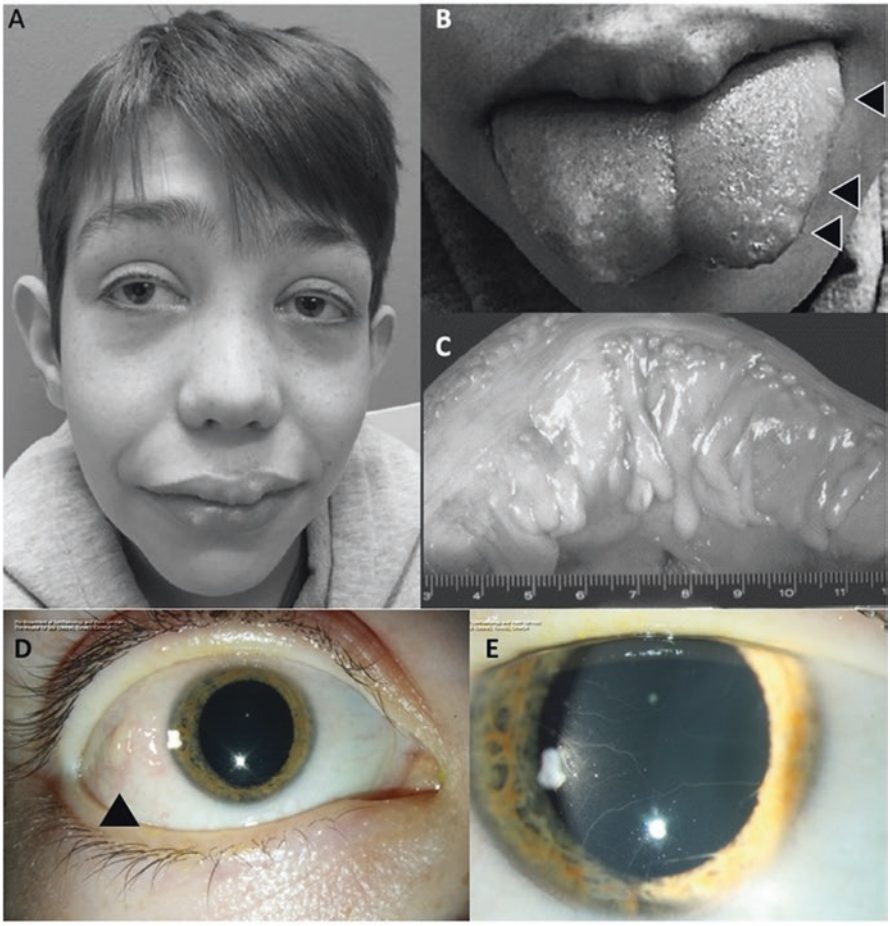

Fig. 8.3 Extra-adrenal phenotypes of MEN 2B include the following: (**a**) Characteristic elongated facies, thickened, everted eyelids and swollen lips due to mucosal neuromas in a young man with MEN 2B. (**b**) Mucosal neuromas (shown here on the tongue). (**c**) Diffuse intestinal ganglioneuromatosis. Reproduced with permission from [120]. (**d**, **e**) Ocular findings of MEN 2B: Again noted are everted eyelids (**d**), conjunctival neuroma (**d**, arrowhead), and corneal neovascularization (**e**). Photos **d** & **e** courtesy of Dr. A. Zhu

8.4.5.2 Pheochromocytoma

Standard treatment for pheochromocytoma is laparoscopic or retroperitoneal adrenalectomy. When bilateral pheochromocytoma is diagnosed, cortical-sparing adrenalectomy is advised to preserve glucocorticoid production [130]. Pre-operative alpha-adrenergic blockade (most commonly with phenoxybenzamine or doxazosin) and, often, beta-blockade are necessary to prevent intra-operative hypertensive crisis. Protocols for pre-operative management of pheochromocytoma have been published [131, 132].

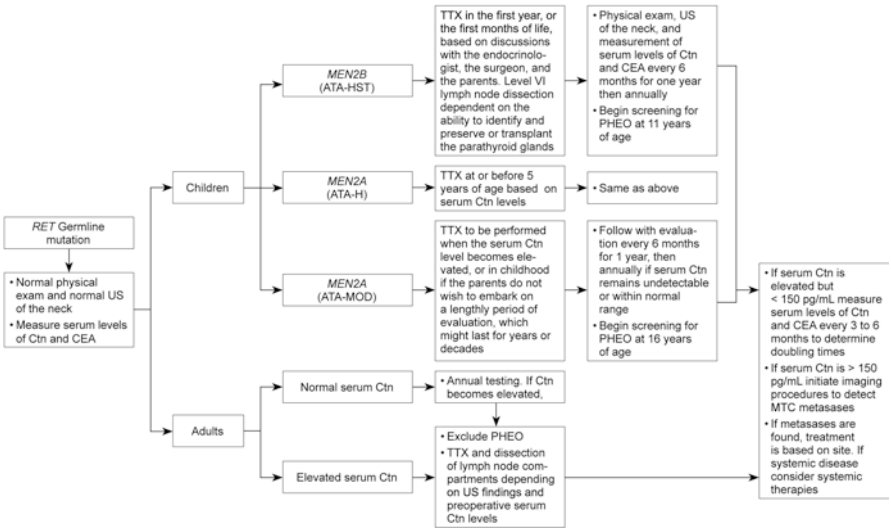

Fig. 8.4 Management of patients with a *RET* germline mutation detected on genetic screening. *ATA* American Thyroid Association risk categories for aggressive medullary thyroid carcinoma (MTC) (HST, highest risk; H, high risk; MOD, moderate risk), *Ctn*, calcitonin, *CEA* carcinoembryonic antigen, *HPTH* hyperparathyroidism, *PHEO* pheochromocytoma, *RET RE*arranged during *T*ransfection, *TTX* total thyroidectomy, *US* ultrasound. Reproduced with permission [123]

Patients with pheochromocytoma should avoid dopamine antagonists and unopposed beta-blockers, to prevent exacerbation of catecholamine excess.

Patients with MEN 2 or other familial PHEO syndromes should be screened prior to planned pregnancy and as soon as possible after unplanned pregnancies.

8.4.5.3 Primary Hyperparathyroidism

In contrast to MEN 1, where total or subtotal thyroidectomy is advocated, patients with PHPT in the context of MEN 2A are advised to undergo minimally invasive parathyroidectomy (MIP) or selective parathyroidectomy with removal only of enlarged glands, utilizing intra-operative PTH monitoring [50, 133–135].

8.4.6 Molecular Genetics of MEN 2A, 2B and FMTC

8.4.6.1 Gene

MEN 2 and FMTC result from variants in the *RET* (*re*arranged during *t*ransfection) proto-oncogene. Variants in *RET* are identified in >98% of patients meeting criteria for MEN 2A or 2B and ~ 95% of patients with FMTC [136]. The *RET* gene is

located at 10q11.2. It encompasses 21 exons and approximately 55 kb of genomic DNA. Alternative splicing at the both the 5′ and 3′ ends creates multiple isoforms [137, 138]. It encodes a membrane-bound receptor tyrosine kinase which is a member of the cadherin superfamily of proteins which share extracellular calcium binding domains involved in adhesion and recognition. The protein is composed of three domains: a cytoplasmic tyrosine kinase domain, a transmembrane domain, and an extracellular ligand-binding domain (Fig. 8.6). The extracellular domain contains four cadherin-like repeats and a highly conserved cysteine-rich region. RET activation requires the formation of a multimer consisting of two RET molecules, two ligand molecules, and two co-receptor molecules (GDNF-family receptor-a (GFRa) protein) [139]. The ligands derive from the glial cell line-derived neurotrophic factor (GDNF) family [140]. The cysteine-rich region of the RET molecule is required for disulfide bonding during multimer formation. Multimer formation results in intracellular kinase activation via autophosphorylation. This leads to the activation of multiple signaling pathways mediating cell motility, proliferation, differentiation, and survival [139].

The *RET* gene is expressed during human embryonic development in the kidney (nephritic duct, mesonephric tubules, and ureteric bud) and neural crest-derived tissues such as the neuroblasts of the developing enteric nervous system, as well as cranial ganglia and spinal cord [141]. After birth, it continues to be expressed in neural and neuroendocrine tissues [142].

8.4.6.2 Genetic Testing

Genetic testing of the *RET* gene is the first step in management planning for suspected cases of MEN 2. It distinguishes sporadic from familial cases and informs management and future surveillance for the patient and family members. Molecular testing is classically initiated with targeted sequencing of select variants (MEN 2B) or select exons (MEN 2A/FMTC) with reflex sequencing of all coding regions and splice junctions, although primary whole-gene sequencing is now widely accessible.

Because of the high penetrance and morbidity associated with undiagnosed MTC in variant carriers, identification of carrier status is critical. *RET* testing is indicated in the following circumstances:

(a) All patients with a clinical diagnosis of MEN 2, primary C-cell hyperplasia, or MTC should be offered testing. More than 98% of patients with MEN 2 and up to 7% of apparently sporadic cases of MTC are found to have an underlying germline *RET* variants [87, 143].
(b) Children of *RET* variant carriers should be offered variant testing, if possible in infancy or childhood depending upon type of variant in the family (Fig. 8.4). Prenatal testing is available, although specific recommendations have not been issued.
(c) Following a diagnosis intestinal ganglioneuromatosis and clinical phenotype consistent with MEN 2B.

(d) Patients diagnosed with cutaneous lichen amyloidosis [87].
(e) In all cases of Hirschsprung disease [87].

8.4.6.3 Genotype/Phenotype Relationships

The majority of variants underlying MEN 2 and FMTC are missense variants that result in a constitutively active RET receptor tyrosine kinase [NM_020975.6]. There are strong genotype-phenotype correlations between individual variants and associated syndromes, age of onset of neoplasm, and with associated symptoms and signs (Fig. 8.6). Age of onset of MTC is based in large part on the transforming potential of the variant [145]. Both age of onset and rate of progression are related to the type of activating germline *RET* variant [94].

Based on these genotype-phenotype correlations, a model has been derived to stratify variants into risk levels and to derive recommendations for the timing of prophylactic thyroidectomy. The most recent American Thyroid Association (ATA) guidelines, published in 2015, divide the variants into three categories, predicated on typical age of MTC onset, highest (HST), high (H), and moderate (MOD) [123], as shown in Table 8.5 and Fig. 8.4.

Variants in exon 10 (codons 609, 611, 618, or 620) or exon 11 (codon 634) account for 98% of MEN 2A (Fig. 8.5). These variants disrupt the extracellular cysteine-rich regions of the RET molecule [146]. Variants causing MEN 2A result in RET molecules which inappropriately dimerize and auto-phosphorylate in the absence of ligand-binding. It is suggested that the disrupted cysteine residues form aberrant disulfide bonds, leading to autophosphorylation and increased activity [147].

FMTC is caused by variants in a subset of those codons associated with MEN 2A, as well as other variants located throughout the gene [87].

In approximately 95% of cases, MEN 2B is attributable to a single missense variant, Met918Thr (exon 16), which causes changes in the substrate-recognition pocket of the intracellular catalytic core [148]. This appears to change the substrate specificity, leading to the phosphorylation of inappropriate substrates [147, 149]. Variants causing the MEN 2B phenotype do not result in inappropriate dimerization.

Table 8.5 American Thyroid Association risk categories for MTC based on *RET* codon. From [47]

ATA MTC risk category	RET codon	MTC	PHEO	PHPT	CLA	HD
Highest (HST)	918	+++	+++	–	–	
High (H)	634, 883	+++	+++	+	+ (only codon 634)	
Moderate (MOD)	All others	+++	+/++	+	+ (only codons 611, 804 and 891)	+ (only codons 609, 611, 618, 620)

Data from Wells et al. [66]. American Thyroid Association (ATA) risk categories based on RET allele: *CLA* cutaneous lichen amyloidosis, *HD*, Hirschsprung disease, *MTC* medullary thyroid carcinoma, *PHEO* pheochromocytoma, *PHPT* primary hyperparathyroidism

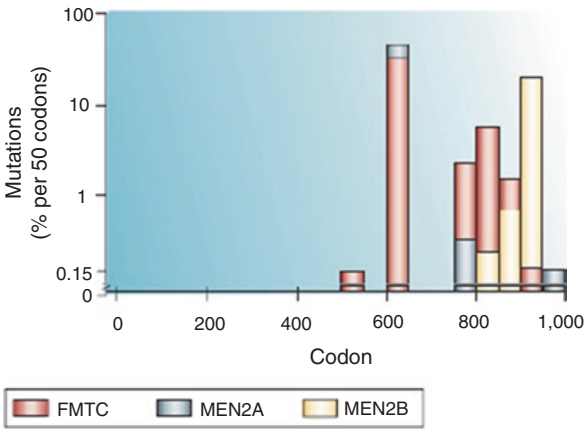

Fig. 8.5 Codon (and syndromic) distribution of *RET* [NM_020975.6] variants. Variants are clustered in distinct regions of the gene, in contrast with variants in *MEN1* (Fig. 8.3). Reproduced with permission [74]

Interestingly, while MEN 2B results in a more aggressive phenotype than MEN 2A, MEN 2B variants do not result in increased kinase activity [147].

8.4.6.4 RET Variants of Uncertain Significance

Although meticulous genotype-phenotype associations have been described, several *RET* variants without clear phenotypic association have been identified. The web-based ARUP online Scientific Resource RET database (http://arup.utah.edu/database/men2/) [150] classifies all reported RET variants as pathogenic, benign, or a variant of uncertain significance (VUS). Currently 102 non-synonymous VUS have been identified (out of 199 total variants). Identification of a VUS poses significant challenges to the clinician vis-à-vis screening and intervention. In the absence of gold-standard functional predictive data, the pathologic potential of a VUS cannot be determined. In an effort to address this, Crockett and colleagues described a predictive algorithm for VUS classification that, when tested against known variants, performed with 93.8% sensitivity and 86.7% specificity, although clinical validation of the VUS predictions is still awaited [151].

8.4.7 Pre-Symptomatic Screening and Surveillance

8.4.7.1 Medullary Thyroid Carcinoma Screening and Surveillance

Biochemical screening with calcitonin (Ct) levels is recommended for pre-symptomatic detection of MTC. Ultrasound is not recommended as a screening modality due to poor sensitivity [152]. Guidelines for timing of initial screening with serum Ct are genotype-dependent (see Fig. 8.4). Use of calcitonin to monitor for disease in young children is complicated by the recognition that calcitonin levels

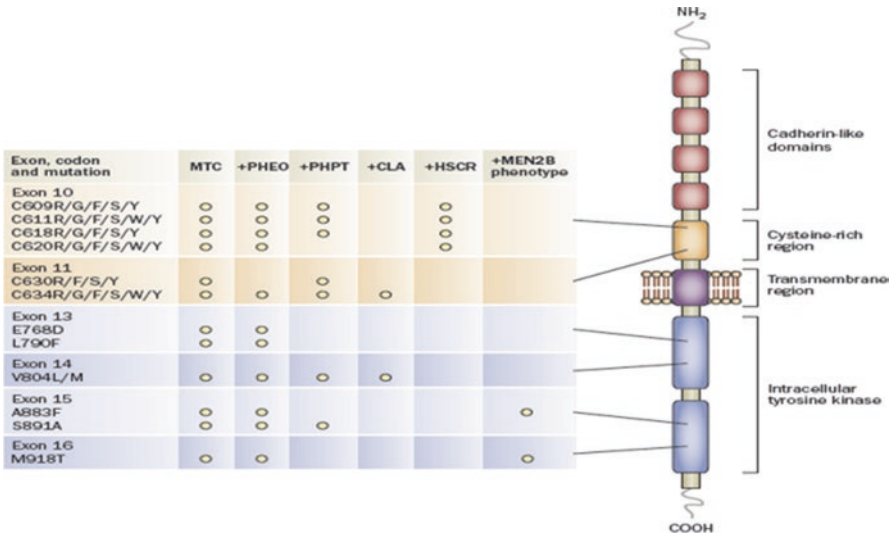

Fig. 8.6 The RET receptor [NM_020975.6] and commonly mutated codons and associated phenotypes in the MEN 2 syndromes. RET is a membrane-bound receptor tyrosine kinase; the extracellular domain contains four cadherin-like domains and a cysteine-rich region, and the intracellular domain contains a tyrosine kinase domain. *Age of testing refers to the age at which clinical testing with thyroid ultrasonography and basal calcitonin levels would be appropriate. It is not meant to indicate the age of testing for the presence of a *RET* variant, which may be done earlier after appropriate genetic counseling. ‡A rare case of very aggressive MTC in a child diagnosed at age 6 years has been reported [144], but in general, an older age of disease onset is observed with this *RET* variant. §Only rare cases of MEN 2B secondary to the A883F variant have been published, and the MTC phenotype of this variant remains largely unknown. Abbreviations: CLA, cutaneous lichen amyloidosis; HSCR, Hirschsprung disease; MEN 2, multiple endocrine neoplasia type 2; MTC, medullary thyroid carcinoma; PHEO, pheochromocytoma; PHPT, primary hyperparathyroidism. Modified from [92]

are higher in infancy and decline through the first 3 years of life and reliable reference ranges for this age group are not always available [153, 154].

Post-operative surveillance of calcitonin and carcinoembryonic antigen (CEA, another MTC tumor marker) levels is recommended every 6 months. Doubling time of less than 2 years of either calcitonin or CEA is associated with decreased recurrence-free survival [155]. An online calculator is currently available for the determination of doubling times (http://www.thyroid.org/thyroid-physicians-professionals/calculators/thyroid-cancer-carcinoma/).

8.4.7.2 Pheochromocytoma Screening

Screening for PHEO is accomplished by measurement of 24-h fractionated urine metanephrines and normetanephrines or plasma free metanephrines and normetanephrines (Table 8.6). Commencement of screening is advocated at age 11 for those with highest (ATA-HST) and high (ATA-H) risk variants and age 16 for those with moderate (ATA-MOD) risk variants [156]. The use of ultrasound for screening

asymptomatic patients is not recommended. There should be a low index of suspicion to screen for PHEO in the presence of symptoms or signs. Additionally, PHEO screening should be performed pre-operatively for all patients and for reproductive-age women prior to planned pregnancies (or as soon as possible in the context of unplanned pregnancies).

8.4.7.3 Primary Hyperparathyroidism Screening

Carriers of MEN 2A-associated variants should undergo annual serum calcium and parathyroid hormone evaluation to detect PHPT. Ages for initiation of surveillance coincide with those for PHEO [156] (Fig. 8.4).

8.5 Multiple Endocrine Neoplasia 4/MENX

Recently, a spontaneous variant arising in a rat colony was noted to result in a phenotype with clinical overlap between MEN 1 and MEN 2 syndromes [157]. Affected animals, referred to as "MENX," develop multiple endocrine tumors including parathyroid adenomas, bilateral pheochromocytoma/paraganglioma, thyroid C-cell hyperplasia, and pancreatic hyperplasia. The syndrome was linked to variants in the *CDKN1B* gene, encoding the $p27^{kip1}$ cell cycle inhibitor. Subsequently, germline variants in *CDKN1B* were identified among patients with clinical similarities to these MENX rats [158]. In humans, this has now been designated as MEN 4, although variants in *CDKN1B* are not universally found in such patients [159]. To date, 19 germline variants have been identified among individuals with multiple endocrine tumors [157, 158, 160–163]. Where tumor tissues exist, these variants appear to result in normal *CDKNIB* mRNA but low or undetectable protein levels. While hyperparathyroidism is found among all affected patients, other tumor types vary between affected individuals. Thus far, the tumor spectrum in humans appears to be exclusively that of MEN 1, and no MEN 2-type tumors have been identified in affected individuals (in contrast to findings in the rat). Germline or somatic *CDKN1B*

Table 8.6 Test characteristics for markers of pheochromocytoma in children and adults. Reproduced with permission [103]

	Sensitivity (%)		Specificity (%)	
Biochemical test	Children	Adults	Children	Adults
Plasma normetanephrine and metanephrine	100	99	94	89
Plasma norepinephrine and epinephrine	92	84	91	81
Urinary normetanephrine and metanephrine	100	97	95	69
Urinary norepinephrine and epinephrine	100	86	83	88
Urinary vanillylmandelic acid	–	64	–	95

Children: adapted from Weise et al. [5] (based on 45 children studied, 12 pheochromocytomas)
Adults: adapted from Zelinka et al. [67] and Lenders et al. [42]

variants were identified among individuals with sporadic PHPT, GEP-NET, lymphoma, and breast carcinoma, expanding the role for *CDKN1B* in other neoplasms (summarized in [163]).

Given the rarity of this diagnosis, and the variability in presentation, no clear clinical definition for MEN 4 has been established. At present, assessment for *CDKN1B* variant is generally restricted to those patients for whom prior *MEN1* testing has been negative.

8.6 Ethical Issues Related to Multiple Endocrine Neoplasias

Diagnosis of a highly penetrant cancer disposition with dominant inheritance carries significant ethical ramifications for both patient and family members. High risk for malignant disease in childhood, as well as recommendation for prophylactic surgery, engenders further complexity to the care of these patients and introduces unique ethical challenges.

Genetic counseling (ideally in a multi-disciplinary context) prior to and following molecular testing is essential in the setting of a possible MEN diagnosis and should be conducted in a carefully considered manner with the expert guidance of clinicians trained in the management of these conditions. A separate chapter in this text examines ethical issues in greater detail; however, we will address some of the core concerns inherent to a diagnosis of multiple endocrine neoplasia including *duty to warn* and the rights of parents to decline testing and/or treatment for their children.

Although there are clear genotype-phenotype correlations in MEN 2A and 2B, allowing reasonable prediction of disease course for affected individuals, MEN 1 presents with a highly variable course, engendering greater ambiguity. Expectant management and surveillance can mitigate morbidity and potentially mortality associated with variant carriage.

Is there a duty to warn? For individuals demonstrated to carry germline variants in *MEN1* or *RET*, first-degree relatives have a 50% risk of variant carriage (unless the proband acquired a de novo variant). This 50% risk of variant carriage also incurs a risk of potentially fatal malignancy (metastatic GEP-NET in up to 75% of MEN 1 carriers and nearly 100% risk of MTC among *RET* carriers). This risk of mortality and morbidity can be dramatically reduced by early detection via presymptomatic screening for known carriers. Patients identified to carry variants should be urged to share these results with family members or, alternatively, offered a mechanism to communicate this risk to relatives, without identifying the index case. In circumstances where individuals decline to share these results, the clinician should explore the reasons for such denial (including social, educational, and ethical barriers, family dynamic, cultural beliefs, etc.). Continued refusal places the clinician in conflict between his/her obligations to protect patient autonomy and

confidentiality and the mandate to protect others from harm or death [164]. Legally, the clinician is similarly conflicted, as privacy legislation (*HIPAA* in the USA or *PHIPA* in Canada) mandates confidentiality, while case law such as *Pate v. Threlkel* [165], in which a surgeon was successfully sued by the daughter of a patient with MTC for failure to warn her of the hereditary nature of her mother's disease, reinforces the duty of the clinician to protect those in imminent or foreseeable risk of harm [166]. The judgment in the latter case concluded that the burden to the clinician would be met by adequately informing the index patient of the heritable nature of her disease and urging her to disclose the results to family members.

Professional societies have failed to reach consensus on the permissibility of violating patient confidentiality to notify family members of genetic risks. The American Society of Human Genetics supports the ethical justification for disclosure in certain situations [167], and this position is endorsed by both the World Health Organization [168] and the National Human Genome Research Institute. On the other hand the American Medical Association and the American Association of Clinical Oncology discourage violation of confidentiality under any circumstance [169–171].

Can parents decline testing/treatment for children? Further ethical conflict arises in situations where parents decline testing or prophylactic surgery for children at risk of MTC. Prophylactic thyroidectomy in MTC has, since its introduction, shifted the approach in predisposed individuals, from cancer treatment to primary prevention. In the case of familial MTC or MEN 2, a positive test for *RET* variant has been suggested to be equivalent in terms of diagnostic accuracy to a positive biopsy [172]. The risk of malignancy, however, is neither immediate nor emergent; thus, the situation must be distinguished from those in which children may be treated without parental consent (such as traumatic resuscitation). As such, the primary responsibility with the clinician is to ensure parental understanding of the diagnosis, implications, and risks and to provide exposure to the appropriate professional consultants (genetic counselors, surgeons, endocrinologists, oncologists, social workers, ethics committees, etc.) to facilitate an informed decision process. In the case of older children, the child should also be included in the decision-making process. Legal recourse should be relegated to a situation of last resort, when concern for medical neglect persists despite the clinician's best efforts.

Finally, in the case of MEN 1, genetic testing of children should be approached cautiously, as the likelihood of actionable complications in childhood is low and the benefit of pre-symptomatic variant screening and surveillance has not been demonstrated; thus, deferral of testing until the child reaches the age of consent may be reasonable, provided appropriate clinical surveillance is instituted.

The issues discussed here have been reviewed in greater detail by Shuman et al. [172], Rosenthal and Diekma [173], and Lips and Höppener [174], and the reader is referred to these citations for further reading.

8.7 Online Resources Related to Multiple Endocrine Neoplasias

8.7.1 Patient Support Groups

MEN
- http://www.amend.org.uk/
- http://www.amensupport.org/

Pheochromocytoma
- https://pheopara.org/
- https://www.tapatalk.com/groups/pheochromocytomasupportboard

Neuroendocrine Tumors
- http://www.netpatientfoundation.org/
- http://www.carcinoid.org/

Medullary Thyroid Carcinoma
- http://www.thyca.org
- http://www.thyroidcancercanada.org/

8.8 Clinician Resources

Practice Guidelines
- https://online.liebertpub.com/doi/full/10.1089/thy.2014.0335
- http://www.nccn.org/professionals/physician_gls/pdf/thyroid.pdf
- https://jamanetwork.com/journals/jamasurgery/fullarticle/2542667

Patient Handouts
- https://www.thyroid.org/medullary-thyroid-cancer/

Pheochromocytoma and paraganglioma RESearch Support ORganization (PRESSOR)
- http://www.pressor.org/

Databases of Genotype-Phenotype Associations
- http://www.arup.utah.edu/database/MEN1/MEN1_welcome.php
- www.umd.be/MEN1/
- http://www.arup.utah.edu/database/MEN 2/MEN2_display.php

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Chapter 9
DICER1 Syndrome

William D. Foulkes, Leanne de Kock, and John R. Priest

Abstract DICER1 syndrome, known previously as the pleuropulmonary blastoma—familial tumor dysplasia syndrome (OMIM #601200, #138800, #180295), was first described clinically in 1996. In 2009, heterozygous pathogenic variants in *DICER1* (OMIM *606241) were found to cause the syndrome now referred to as DICER1 syndrome; since then, numerous investigations have revealed that more than 25–30 phenotypes comprise DICER1 syndrome. The phenotypes are mostly rare to ultra-rare malignant and benign proliferative lesions, which occur from birth through ages 30–40 years. DICER1 syndrome is notably pleiotropic, but the most frequent and distinctive disorders are pleuropulmonary blastoma, cystic nephroma, and ovarian Sertoli-Leydig cell tumors, yet each has disease penetrance under 10%. In contrast, multinodular goiter, the least specific DICER1 phenotype, has penetrance approaching 75% in females and 20% in males. Other rare and highly characteristic conditions include pituitary blastoma, embryonal rhabdomyosarcoma of the uterine cervix, anaplastic renal sarcoma, as well as rare ocular and sinonasal tumors. Numerous reports of unusual rhabdomyosarcomatous tumors in young

William D. Foulkes, Leanne de Kock, and John R. Priest contributed equally to this work.

W. D. Foulkes (✉)
Department of Medical Genetics, McGill University Health Centre and Jewish General Hospital, Montreal, QC, Canada

Departments of Human Genetics, Oncology and Medicine, McGill University, Montreal, QC, Canada

Segal Cancer Centre, Lady Davis Institute, Jewish General Hospital, Montreal, QC, Canada

Research Institute, McGill University Health Centre, Montreal, QC, Canada
e-mail: william.foulkes@mcgill.ca

L. de Kock
Segal Cancer Centre, Lady Davis Institute, Jewish General Hospital, Montreal, QC, Canada

Department of Human Genetics, McGill University, Montreal, QC, Canada

Research Institute, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada
e-mail: leanne.dekock@mail.mcgill.ca

J. R. Priest
Minneapolis, MN, USA

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individuals, arising in the brain or in abdominal spaces, have *DICER1* variants as the cause and reveal similar and characteristic histopathology. Conditions such as pineoblastoma, Wilms tumor, and juvenile hamartomatous intestinal polyps may also occur but do not on their own suggest DICER1 syndrome.

The predisposing *DICER1* alterations are typically pathogenic loss-of-function variants in the germline. Termination and frameshift variants are common, but large and small deletions are also seen; mosaicism also causes DICER1 syndrome. In addition, most DICER1-related tumors harbor a highly characteristic somatic mutation in the second *DICER1* allele impairing DICER1 protein's RNase IIIb endonuclease function, which normally cleaves precursor microRNAs to their mature length. MicroRNAs function by targeted silencing and/or post-transcriptional degradation of specific messenger RNAs. Thus, *DICER1* has emerged as an unusual tumor suppressor gene: the first molecular "hit" cripples one allele completely, whereas the somatic second "hit" is a single base substitution leading to an amino acid change in the RNase IIIb cleavage domain. This impairs the function of the protein, without overall protein loss, leading to unbalanced microRNA products.

Keywords DICER1 · Pleuropulmonary blastoma · Embryonal tumors · MicroRNA · Pediatric cancer · Development · Sertoli-Leydig cell tumor · Cystic nephroma · Multinodular goiter · Pituitary blastoma · Pineoblastoma · Anaplastic sarcoma of the kidney

9.1 Introduction

Heterozygous pathogenic variants in the critical microRNA (miRNA) processing gene *DICER1*, located at chromosome 14q32.13, underlie the distinctive DICER1 syndrome—a childhood tumor and dysplasia syndrome recognized in recent years [1–5]. Such variants are present most often in the germline and are usually inherited [6]. Deletions of *DICER1* and mosaicism also cause the syndrome [7–10]. Approximately 25–30 phenotypes have been reported to date (Fig. 9.1, Table 9.1); rare phenotypes will continue to be identified. Mesenchymal proliferations, both malignant and benign, are typical. Pleuropulmonary blastoma (PPB), an early childhood sarcoma of lung and pleura, is the hallmark disease [11, 12], along with several other characteristic conditions, such as ovarian Sertoli-Leydig cell tumors, cystic nephroma, and rhabdomyosarcoma of the uterine cervix (Fig. 9.1, Table 9.1). DICER1-related sarcomas in diverse anatomic sites including cerebrum, kidneys, pelvis, and other sites are also characteristic of DICER1 disease and remarkably similar pathologically to PPB as discussed below (Fig. 9.1, Table 9.1) [13–17].

DICER1 syndrome exhibits marked pleiotropy and has low penetrance, generally less than 10% for any phenotype other than multinodular goiter (MNG) and

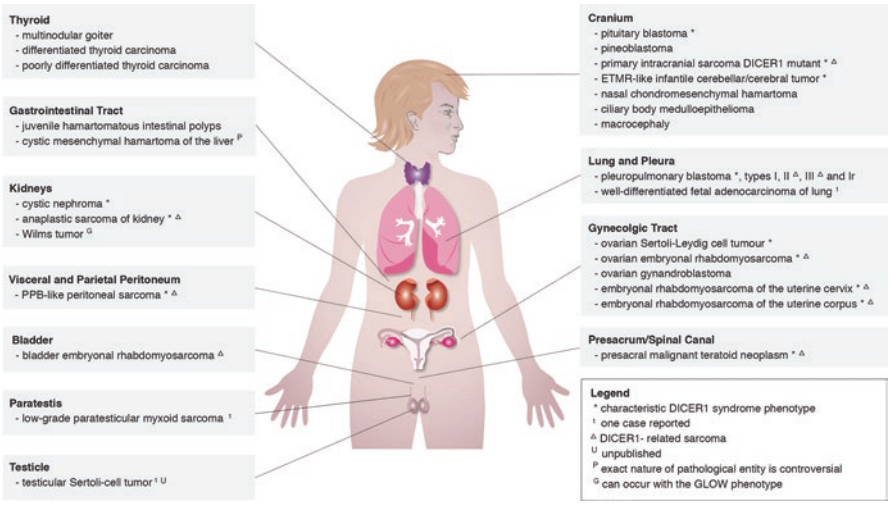

Fig. 9.1 DICER1 syndrome phenotype. Diseases recognized in *DICER1* variant carriers and in kindred clinically manifesting the DICER1 syndrome. Germline and somatic *DICER1* alterations have been demonstrated in each condition. This is an update and re-organization of a previously published figure [190]. Abbreviations: ETMR embryonal tumor with multilayered rosettes; PPB - pleuropulmonary blastoma

occult lung cysts. Detailed studies of affected cohorts have revealed that clinically or radiographically detected thyroid nodules affect three-quarters of females and up to 17% of males carrying pathogenic variants by age 40 years [18]. Similarly, occult often small lung cysts detectable by computed tomography are found in 25–30% of carriers [19]. The gynecologic manifestations and greater frequency of MNG in females compared to males result in higher overall penetrance in females. Bilateral disease in paired organs is not unusual. As shown in Table 9.1, some diseases in the syndrome have highly focused ages of presentation.

The typically adult-onset cancers found in certain other childhood and adolescent tumor predisposition syndromes, such as the Li-Fraumeni syndrome, do not appear to be part of the DICER1 complex. No evidence has emerged to date that DICER1 syndrome is more or less prevalent in any ethnic or racial group. There is also no evidence to date that *DICER1* variant carriers are prone to developing second malignant neoplasms as a result of cancer therapies such as alkylating agents or therapeutic radiation. The *possible* exception to this, discussed later in this chapter, is development of thyroid carcinoma as a result of intensive multimodal therapies such as stem cell transplant for a serious DICER1 disease or as a result of high cumulative diagnostic radiation exposures from chest computed tomography, as often occurs following a PPB diagnosis.

DICER1 is a cytoplasmic endoribonuclease III which cleaves hairpin precursor pre-miRNAs, produced in the cell nucleus by similar enzymes such as DROSHA (RNASEN) and DGCR8 (PASHA), into mature, non-coding regulatory miRNAs comprised of ~23 base pairs. As part of the RNA-induced silencing complex,

Table 9.1 DICER1 syndrome phenotypes and their typical ages of presentation, specificity for DICER1 syndrome, and biologic behavior

Phenotype[a]	Typical ages of presentation m, months y, years	Characteristic of DICER1 syndrome and genetic testing indicated	Typical biologic behavior B, benign M, malignant U, uncertain biologic behavior [hc] high cure potential [mc] moderate cure potential [lc] low cure potential [uncertain] uncertain prognosis
Lung and pleura diseases			
Pleuropulmonary blastoma			
Type I (cystic)	0–2y	Yes	M[hc]
Type II (cystic/solid)[b]	6 m–4 y	Yes	M[mc]
Type III (solid)[b]	1–6 y	Yes	M[mc]
Type Ir (cystic)	Any age	Yes	B
Well-differentiated fetal adenocarcinoma of the lung	Not established	No	M[hc]
Thyroid disease			
Multinodular goiter	3–any	No	B
Differentiated thyroid carcinoma	8–any	No	M[hc]
Poorly differentiated thyroid carcinoma	Not established	Yes	M[hc]
Renal disease			
Cystic nephroma	0–48 m	Yes	B
Anaplastic sarcoma of the kidney[b]	1–20 y	Yes	M[mc]
Wilms tumor	1–10 y	No	M[mc]
Gastrointestinal disease			
Juvenile hamartomatous polyps	0–20 y	No	B
Cystic mesenchymal hamartoma of the liver[c]	0–4 y[d]	No	B
Cranial disease			
Pituitary blastoma	0–24 m	Yes	U[mc]
Pineoblastoma	2–20 y	Only if <10 y	M[mc]
Primary cerebral sarcoma—*DICER1* mutant[b]	1–20 y[d]	Yes	M[mc]
ETMR-like infantile cerebral/cerebellar embryonal tumor	<36 m	Yes	M[lc]
Nasal chondromesenchymal hamartoma	6–20 y	No	B

(continued)

Table 9.1 (continued)

Phenotype[a]	Typical ages of presentation m, months y, years	Characteristic of DICER1 syndrome and genetic testing indicated	Typical biologic behavior B, benign M, malignant U, uncertain biologic behavior [hc] high cure potential [mc] moderate cure potential [lc] low cure potential [uncertain] uncertain prognosis
Ciliary body medulloepithelioma	3–10 y	No	B, rare $M^{uncertain}$
Gynecologic diseases			
Ovarian Sertoli-Leydig cell tumor	2–50+ y[e]	Yes	M^{hc}
Ovarian gynandroblastoma	10–25 y[d]	Yes	M^{hc}
Ovarian rhabdomyoblastic sarcoma[b]	5–15 y[d]	Yes	$M^{uncertain}$
ERMS of the uterine cervix[b]	5–25 y	Yes	M^{hc}
ERMS of the uterine corpus[b]	10–25 y[d]	Yes	M^{hc}
PPB-like sarcoma of peritoneum including fallopian tube serosa[b]	10–20 y[d]	Yes	M^{mc}
Bladder diseases			
ERMS bladder[b]	3–15 y[d]	No	M^{hc}
Pelvic/abdominal cavity and serosal diseases			
Presacral malignant teratoid tumor[b]	<5 y[d]	Yes	$M^{uncertain}$
PPB-like peritoneal sarcoma[b]	3–15 y[d]	Yes	M^{mc}
Non-proliferative manifestations of DICER1 syndrome			
Macrocephaly	Any	No	Not applicable
Dental dysmorphologies	Any	No	Not applicable
Ocular abnormalities	Any	No	Not applicable
Kidney and collecting system dysmorphologies	Any	No	Not applicable
"GLOW" phenotype			
Includes several manifestations including global developmental delay, overgrowth, lung cysts, Wilms tumor, and other findings. See references [20, 36, 37]			

Abbreviations: *ERMS* embryonal rhabdomyosarcoma; *PPB* pleuropulmonary blastoma
[a]Nomenclature to describe rare and recently reported phenotypes subject to change, especially for DICER1-related sarcomas in unusual sites
[b]DICER1-related sarcoma
[c]Exact nature of pathological entity is controversial
[d]Age range approximated when few cases known
[e]95% diagnosed under age 40 years [191]

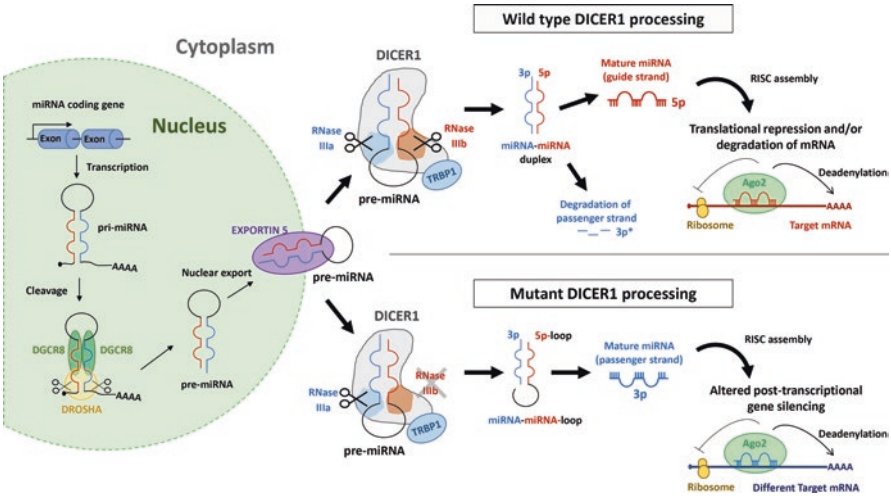

Fig. 9.2 Canonical pathway of miRNA biogenesis. Most miRNA genes are transcribed by RNA polymerase II in the nucleus. Then, primary miRNAs (pri-miRNAs) are cleaved by the microprocessor complex, formed by DROSHA and DGCR8, into precursor miRNAs (pre-miRNA), which are subsequently exported to the cytoplasm by Exportin 5. In the cytoplasm, DICER1 cleaves pre-miRNAs into mature microRNAs (3p-5p duplex). The 5p strand is derived from the 5′ end of the pre-miRNA hairpin, while the 3p strand originates from the 3′ end. The resulting miRNA-miRNA duplex is unwound by a helicase, and while the passenger strand is cleaved and degraded, the guide strand (usually the 5p) is loaded onto the miRNA-induced silencing complex (miRISC) to target mRNAs for post-transcriptional gene silencing [wild-type DICER1 panel]. However, in cells expressing a mutant DICER1 that lacks a functional RNase IIIb catalytic domain, the maturation of 5p strand is impeded, whereas the 3p strand is properly processed. Therefore, only the 3p strand will be loaded into miRISC to target mRNAs [mutant DICER1 panel]

DICER1, mature miRNAs, and other co-factors target specific messenger RNAs for post-transcriptional downregulation, thereby modulating cellular protein production [1]. A schematic figure of the miRNA biogenesis pathway is shown in Fig. 9.2. DICER1 is comprised of numerous domains (Fig. 9.3) including helicase, PAZ, and RNase III domains. As illustrated in Fig. 9.3, the ~254 reported distinct predisposing *DICER1* alterations (pathogenic variants including large and small deletions) occur throughout the gene. Most are loss-of-function variants, and are inherited rather than de novo [6]. Mosaicism appears to cause 4–5% of syndrome cases [7, 8, 20].

In addition to a primary *DICER1* alteration disabling one allele, most tumors in the syndrome exhibit a highly distinctive somatic change in the second *DICER1* allele affecting a narrow set of RNase IIIb metal-ion binding sites of DICER1 protein (Fig. 9.3) [21–28]. The somatic alterations are termed "hotspot" mutations. The RNase IIIb change caused by a hotspot mutation neither fully abrogates DICER1 function nor results in loss of the protein but instead alters the proportions of 3′- (3p) and 5′-derived (5p) miRNAs produced by the protein [1, 29, 30] (Fig. 9.2).

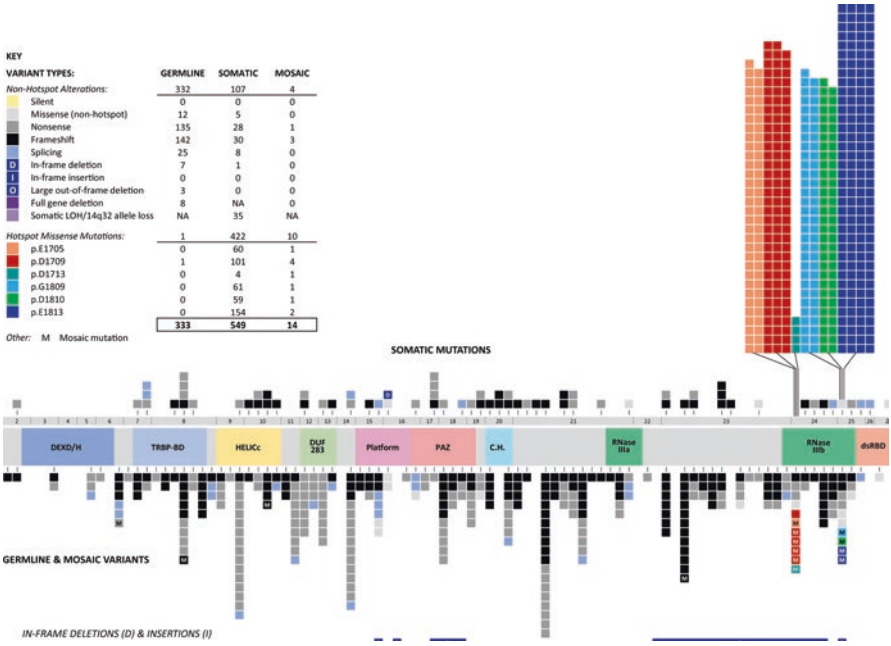

Fig. 9.3 Germline and somatic variants in *DICER1*. Plotted along the length of the unfolded DICER1 protein are all pathogenic and likely pathogenic *DICER1* alterations published prior to June 2020. Germline variants are plotted once per family (unique per family, UPF). A total of 333 UPF germline variants and 14 mosaic variants are plotted below the protein. The 549 confirmed-somatic events are plotted above the protein, except for the 35 confirmed-somatic allele loss events that are shown at the bottom of the figure. DICER1 domains are defined as follows: *DExD/H* DExD/H box helicase domain; *TRBP-BD* trans-activating response RNA-binding protein binding domain; *HELICc* helicase conserved C-terminal domain; *DUF283* domain of unknown function; Platform, platform domain; *PAZ* polyubiquitin-associated zinc finger domain; *c.h.* connector helix; *RNase IIIa* ribonuclease IIIa domain; *RNase IIIb* ribonuclease IIIb domain; *dsRBD* double-stranded RNA-binding domain. Abbreviations: *LOH* loss of heterozygosity; *NA* not applicable. This is an update of a previously published figure [6]

Instead of a somatic RNase IIIb mutation, loss of heterozygosity (LOH) of the wild-type *DICER1* allele is found in some syndrome tumors (in pineoblastoma particularly and in pituitary blastoma) [31]. Because several tumors in DICER1 syndrome occur in relatively tightly defined age ranges in young children (Table 9.1), it appears as if *DICER1* and miRNAs have critical time- and tissue-specific effects on development of certain organs. Examples of this phenomenon are PPB in children under age 6 years, cystic nephroma in children under age 4 years, and pituitary blastoma in children under age 2 years.

Reports to date suggest genotype-phenotype correlations in DICER1 syndrome only for unusual and specific gene alterations. Compared to loss-of-function (LOF) variant carriers, children with mosaic RNase IIIb mutations are diagnosed at significantly younger ages and develop more diseases per affected individual [7, 8]. The

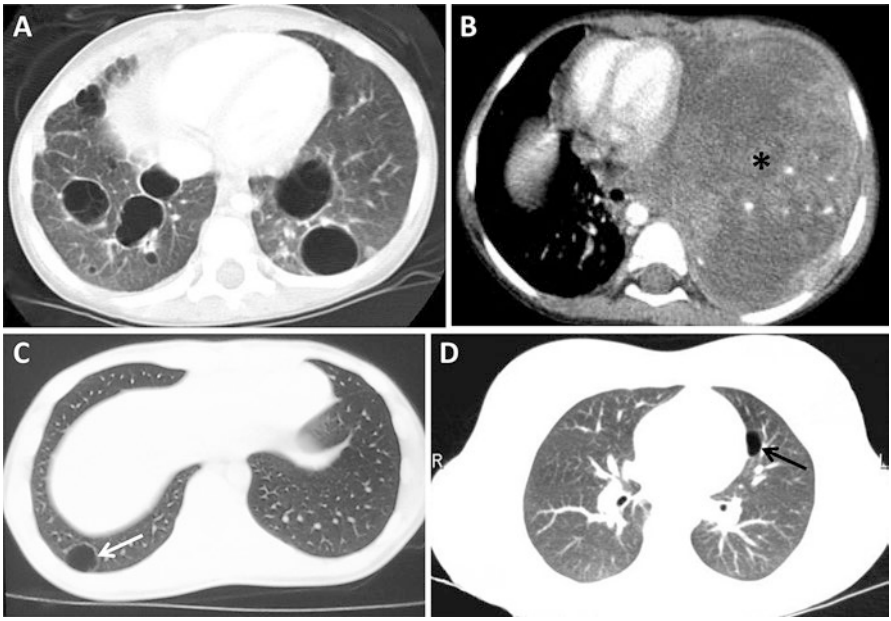

Fig. 9.4 Pleuropulmonary blastoma. (**a**) An axial chest computed tomography (CT) image of a child with a mosaic RNase IIIb hotspot predisposing *DICER1* alteration showing numerous large and small air-filled cysts in the right and left lungs. The patient was subsequently diagnosed with bowel polyps at age 9 months, PPB Type II at 2.1 years, nasal chondromesenchymal hamartoma at 8 years, and metastases of PPB to the brain at ages 2.1 years, 3.1 years, 10.5 years, and 10.9 years. (**b**) An axial chest CT image with black asterisk indicating Type III PPB filling the hemithorax. (**c** and **d**) Axial chest CT images in which small arrows identify occult air-filled lung cyst in variant carriers, which likely represent Type Ir PPB. Patient C developed an esophageal juvenile hamartomatous polyp and multinodular goiter and has a family history of DICER1 syndrome. Patient D was diagnosed with multinodular goiter at age 12 years and had an older sister with DICER1 syndrome, which occasioned chest imaging. (Images courtesy of Barbara Pasini, M.D. [C] and the patient [D])

triad in very young infants of lung cysts, renal cysts, and small bowel polyps may suggest mosaicism [7, 8, 32–35]. Furthermore, these children appear to have unusually abundant lung cysts, often in many if not all lobes bilaterally [20, 22, 32] (Fig. 9.4a). In addition, a very rare complex phenotype of DICER1 syndrome, termed "GLOW," has been identified in two children with mosaic RNase IIIb mutations and one child with a germline RNase IIIa variant (Table 9.1) [20, 36, 37]; GLOW signifies global developmental delays, lung cysts, regional somatic overgrowths including macrocephaly, and Wilms tumor, in addition to other findings.

Biallelic *DICER1* tumor-only alterations in DICER1 syndrome-associated tumors have also been reported [7, 38]. Detailed studies must prove the limited nature of the variants, yet their elucidation is of great benefit to patient and family [38]; because the genetic alteration is restricted to the tumor, such cases are not considered to involve the child or family with DICER1 syndrome.

The DICER1 syndrome should be strongly considered in a child or family with even one of the highly distinctive diseases (Fig. 9.1, Table 9.1) or, because of the syndrome's pleiotropy and low penetrance, in a child or kindred with any combination of the related diseases.

The recent wide utilization of genetic sequencing of many human tumors has revealed that *somatic* mutations in the family of miRNA processing genes (*DROSHA*, *DGCR8*, *DICER1*, *XPO5*, and *TARBP2*) play a role in human neoplasia [39–41]. A pathogenic variant in *DGCR8* is the only other reported heritable alteration in an miRNA biogenesis gene associated with tumor development; individuals in three generations of one kindred were affected by MNG and schwannomatosis [42]. In addition, childhood tumors similar to DICER1-related tumors are associated with miRNA disturbances not linked to miRNA biogenesis. Specifically, amplification of the chromosome 19 microcluster (*C19MC*: chr19q13.41 miRNA cluster), amplification of the *miR-17-92* miRNA cluster on chromosome 13 (also known as *MIR17HG*), and *DICER1* alterations cause similar-appearing aggressive early childhood central nervous system tumors termed embryonal tumors with multilayered rosettes, which include tumors previously labeled ependymoblastoma or medulloepithelioma [43, 44]. Both *DICER1* alterations and *C19MC* amplification are associated with similar childhood cystic hepatic mesenchymal lesions [45–47].

We do not discuss in this chapter those diseases in which exclusively somatic *DICER1* mutations are implicated. *DICER1* mutations in the broadest sense have been reviewed elsewhere [6].

9.2 DICER1-Related Sarcomas

In 1988, PPB became the first and remains the hallmark malignant mesenchymal tumor in DICER1 syndrome, but now a wide anatomic distribution of pathologically similar sarcomas is recognized as a characteristic syndrome phenotype [12]. These DICER1-related sarcomas arise in the lung and pleura, cerebrum, cerebellum, kidney, ovary, uterus, and bladder. In addition, similar DICER1-related sarcomas arise in less well-defined abdominal and pelvic sites apparently in visceral and parietal serosa (fallopian tube, presacrum) [13–17]. Unfortunately, as tumors with similar histologies have been identified in various sites, their naming has been diverse and obscures the striking pathological similarities [48]. The DICER1-related sarcomas are identified in Table 9.1 and Fig. 9.1 and are discussed below. In viscera with a lumen (e.g., vagina, bladder, lung cyst), DICER1-related sarcomas characteristically form the grape-like clusters of sarcoma botryoides.

Even before these tumors could be unified by *DICER1* causation, their histologic similarity to PPB was recognized. Pathologists have remarked that some of these tumors could be considered PPB except that they did not arise in the lung [49]. Although not every DICER1-related sarcoma expresses all characteristic pathologic elements, the features include cyst formation, subtle subepithelial malignant mesenchymal cells (small blue cells which may condense into subepithelial "cambium"

layers), malignant stromal and spindle cell areas, skeletal muscle differentiation or de-differentiation with rhabdomyoblastic or embryonal rhabdomyoblastic areas (with myogenin- and myoD-positive immunostaining), blastema, areas of sometimes striking anaplasia, primitive or overt and sometimes malignant cartilaginous and rarely osteoid differentiation, and, very rarely, primitive neuroectodermal elements. Notably absent in these tumors is any epithelial differentiation.

Specific phenotypes are discussed below based on body region as in Fig. 9.1.

9.3 Chest

9.3.1 Pleuropulmonary Blastoma

Pleuropulmonary blastoma is a rare malignant pleural and/or parenchymal lung tumor presenting in children most often under 72 months of age [11, 12, 50, 51]. Several hundred cases have been recognized, predominantly in the collection of the International PPB Registry (IPPBR) (www.ppbregistry.org) [19, 50].

Three malignant manifestations of PPB are recognized along an age and degree-of-malignancy spectrum: cystic Type I PPB in newborns and infants and cystic/solid Type II and solid Type III PPB in progressively older children. These comprise, respectively, 33%, 38%, and 29% of PPB cases [50]. In addition to these malignant PPB types, a non-malignant cystic manifestation of PPB termed Type Ir (regressed) PPB is discussed below. The radiographic appearances of PPB have been reviewed in detail [4, 52].

From birth through approximately age 2 years, cystic Type I PPB is an early malignant lesion presenting with dyspnea or as an incidental finding on a chest radiograph done for other reasons. Radiographically an innocuous-appearing air-filled multilocular lung cyst is noted, often with pneumothorax. The clinico-radiographic features mimic congenital cystic adenomatoid malformation (CCAM), which is a much more frequent disease and indeed is the pre-operative diagnosis in almost every case of Type I PPB [4]. However, PPB instead of CCAM should be suspected when a child has pneumothorax, multifocal or bilateral lung cysts, or a family history of any condition related to *DICER1* mutation (Fig. 9.1; Table 9.1) [4]. To differentiate Type I PPB from CCAM (especially from CCAM type 4 which mimics Type I PPB), expert pathological examination of a resected cyst is essential to identify the often subtle, scattered population of primitive rhabdomyomatous cells in cyst walls and septa; small nodules of immature cartilage are frequent and highly characteristic of PPB [11, 53, 54]. Delicate septations in cysts may not be appreciated in plain radiographs or computed tomography but are highly characteristic [52]. Type I PPB is cured in 90–95% of cases [33, 50].

In children from approximately ages 2 through 6 years, Type II and III PPB occur and are aggressive sarcomas with overall 5-year survival rates of approximately 70% and 50%, respectively [50]. The solid elements of Type II and III PPB

(Fig. 9.4b) express the DICER1-related mixed sarcoma patterns discussed earlier. These advanced forms present commonly as "pneumonia," with dyspnea, cough, malaise, and/or fever (occasionally with pneumothorax for Type II). Although PPB is increasingly recognized, it remains a specialized pathologic diagnosis; among cases submitted to IPPBR review pathologists, 20% are judged not to be PPB [50]. In addition, PPB is an exclusively mesenchymal tumor to be distinguished from biphasic mesenchymal and epithelial "pulmonary blastoma" which has not been reported in DICER1 syndrome [11].

It is well established that Type I PPB in a young child may progress over 1–5 years to Type II or III disease as the scant malignant population overgrows cyst walls and septa [4, 33, 53]. This phenomenon raises the possibility of early detection and possible resection of PPB cysts in variant-carrying infants in known DICER1 families [55, 56].

Type Ir PPB is a non-malignant cystic manifestation of PPB. It is thought to represent either forme fruste or regressed Type I PPB. The key difference from Type I PPB is the absence of a primitive cell population [4, 50, 53]. Type Ir PPB cysts tend to be 2–3 cm in diameter (sometimes larger) and are typically discovered in *DICER1* variant carriers at any age in radiographic studies done for other reasons (Fig. 9.4c and d) [52]. Radiographically, a Type Ir PPB is indistinguishable from a small Type I PPB. In a systematic survey of variant carriers, Type Ir PPB was discovered by computerized tomography in approximately 25–30% of carriers [19]. Because these cysts do not harbor primitive cells and because they have been discovered in adult variant carriers well beyond the ages typical for PPB cyst progression, Type Ir PPB cysts are thought not to have malignant potential. In a variant carrier, cyst progression beyond the age of approximately 8 years is considered highly unlikely, although rare exceptions exist [57, 58]. Currently in an adult variant carrier, a lung cyst can be presumptively diagnosed as Type Ir PPB without resection or pathologic examination. Type Ir PPB is also diagnosed in infants, and progression has been observed [50, 59]; such progression in young children suggests that the scattered malignant cells of Type I PPB may be missed even in detailed pathologic examination or may remain in some areas of a cyst specimen despite evidence of regression elsewhere.

Germline *DICER1* mutations are described in 65–70% of 126 reported PPB patients; somatic *DICER1* mutations, predominantly affecting RNase IIIb hotspots, are described in 94% of 64 PPB tumors [5, 21, 50, 60–63].

In one report, PPB has been noted in association with neurofibromatosis type 1 [64], and in another case report, NF1 was associated with pulmonary blastoma [65], which also contains somatic *DICER1* mutations [66]. Detailed molecular studies have not been reported, and the possible mechanistic connection between *DICER1* and *NF1*, and their associated syndromes, is unknown.

9.3.2 *Well-Differentiated Fetal Lung Adenocarcinoma (WDFLA)*

A single case of WDFLA in the context of DICER1 syndrome has been reported (see "Rare or Possible Associations").

9.4 Head and Neck

9.4.1 *Multinodular Goiter and Other Non-toxic Thyroid Diseases*

Thyroid disease occurring in childhood, adolescence, or early adulthood usually in the form of nodular hyperplasia and often progressing to frank MNG is the most frequent manifestation of a germline *DICER1* pathogenic variant (Fig. 9.5a); by age 40 years, three-quarters of at-risk females and one in six at-risk males will develop MNG or undergo thyroidectomy [18]. As early as the 1950s, there were reports of familial MNG occurring with other DICER1-related phenomena [67], but it was not until 1974 that a direct genetic link between Sertoli-Leydig cell tumor (SLCT)—a type of sex cord stromal (non-epithelial) ovarian tumor—and thyroid adenoma was postulated [68]. Although thyroid disease in general and MNG in particular are frequent in the general population [69], familial MNG is relatively unusual. One very large pedigree was linked to chromosome 14q32 in 1997 [70]. An almost completely penetrant missense mutation in *DICER1* was later identified in the family, confirming that MNG is a DICER1-related phenotype [71]. Molecular studies of DICER1-related MNG have revealed that individual nodules harbor distinct RNase IIIb hotspot mutations suggesting discrete clonal origins of nodules comprising MNG [18, 72–74].

Individuals with DICER1 syndrome have an approximately 16-fold increased risk of differentiated thyroid carcinoma (DTC) compared to Surveillance, Epidemiology and End Results (SEER) rates [18] hypothesized to be due to increased prevalence of premalignant thyroid lesions in heterozygotes [18, 73]. However, the overall contribution of *DICER1* mutations both to familial MNG and to familial DTC appears to be very small [23, 70, 71, 75]. Analysis of *DICER1* is probably not justified in such families unless there are other phenotypes suggesting *DICER1* mutation. An exception could be made in the case of childhood- or adolescent-onset familial MNG/DTC or when involving a male under approximately 18 years of age with MNG/DTC [18]. DICER1-related MNG is predominantly diagnosed between ages 10 and 30 years [18, 71, 76]; DICER1-related DTC also occurs at similar ages but is much less frequent than MNG [18, 19]. Several DTCs have occurred in children intensively treated for PPB and other tumors raising the possibility of a causal link between *DICER1* mutation and intensive multimodal therapy [23, 77, 78]. However, DTC also occurs in *DICER1* heterozygotes in

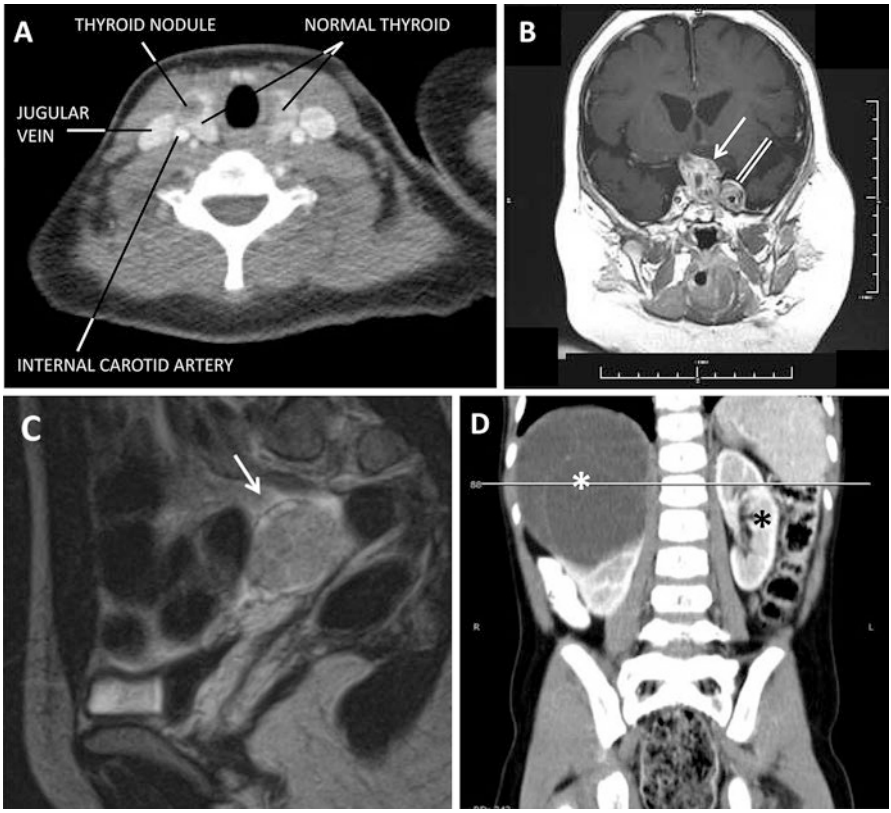

Fig. 9.5 Radiology images. (**a**) Axial cervical region contrast-enhanced CT image of multinodular goiter. (**b**) Coronal gadolinium-enhanced magnetic resonance image of pituitary blastoma (white arrow) with left cavernous sinus involvement (double white line). (**c**) Sagittal pelvic region CT image showing ovarian Sertoli-Leydig cell tumor (arrow). (**d**) Coronal contrast-enhanced abdominal CT image showing multilocular fluid-filled mass pathologically proven to be cystic nephroma in the upper pole of right kidney (white asterisk) above residual normal right lower pole; normal left kidney indicated by black asterisk. (Images courtesy of the patient [A], Marek Niedziela, M.D. Ph.D. [C], and Yves Heloury, M.D. FRACS [D])

the absence of prior treatments [72, 79]. Given their low propensity for metastasis, it is postulated that DICER1-related DTC may form a low-risk subgroup [74, 80]. On the other hand, a recent study identified young-onset clinically aggressive poorly differentiated thyroid carcinoma to be a rare manifestation of DICER1 syndrome [81].

A case of medullary thyroid carcinoma in a patient with a germline *RET* alteration has been reported to harbor two somatic *DICER1* mutations [82]. Eight cases of malignant teratoid tumors of the thyroid have been reported to bear one or more somatic *DICER1* mutations [83–85]. Neither of these tumors has yet been reported in association with germline *DICER1* pathogenic variants.

9.4.2 Cranial and Intracranial Tumors

Because the central nervous system (CNS) is the site of multiple DICER1 syndrome phenotypes including PPB metastases and several primary intracranial tumors, careful diagnosis is essential.

9.4.2.1 Metastasis of Pleuropulmonary Blastoma to the Central Nervous System

Metastasis of PPB to the cerebral hemispheres complicates the course of approximately 7% of Type II PPB cases and 15% of Type III PPB cases and is the most frequent intracranial pathology in DICER1 syndrome [50, 86]. The metastases tend to occur in the first 36 months after chest diagnosis, often without chest recurrence, and may be multiple, fulminant, and recurrent [86]. Cerebral PPB metastasis is also rarely present at the time of chest diagnosis [50]. Cure is possible in perhaps 20% of cases [86, 87]. The pathologic features of the metastases tend to be less complex than the original PPB with predominant rhabdomyoblastic or spindle cell elements consistent with a DICER1 sarcoma as discussed earlier. In fact, the differential diagnosis of PPB metastasis to the brain includes primary cerebral sarcoma—DICER1 mutant [14, 86]. In confusing cases, molecular studies of both chest and cerebral tumors might differentiate metastasis from primary sarcoma in that a metastasis will involve the same *DICER1* hotspot mutation as the chest primary, whereas a primary sarcoma might, though not necessarily, harbor a different hotspot mutation. Leptomeningeal and spinal PPB metastases are extremely rare [86].

Pleuropulmonary blastoma can also affect the cerebrum following a chest resection with tumor embolism causing both hemorrhagic and occlusive vascular events and both early and late tumor growth at the embolic site [86]. Glioblastoma multiforme has occurred in one child 4 years following radiation for PPB CNS metastasis [Sciot, personal communication].

9.4.2.2 Pituitary Blastoma

Pituitary blastoma is an extremely rare, primitive tumor of the anterior pituitary described in one child in 2008 with five confirmatory cases in 2012 [88, 89]. The disease is reported only in children under 24 months of age, and the hallmark symptom is Cushing syndrome, which is otherwise extremely rare in this age group (Fig. 9.5b). Predisposing *DICER1* alterations and/or classic hotspot changes were reported in 2014 in 12 of 12 fully studied cases [22, 90]. Among 17 pituitary blastoma cases now reported, 16 have DICER1 involvement (15 with molecular evidence; 1 with strong clinical evidence of DICER1 syndrome; molecular studies were not possible in 1 case without clinical evidence of DICER1 syndrome) [22, 90–93]. There is a brief report of an 18th case of pituitary blastoma in a 19-year-old

woman [94], which has since been determined to harbor two *DICER1* alterations (Foulkes et al, unpublished data). Thus, pituitary blastoma is virtually pathognomonic for DICER1 syndrome. Despite the blastoma label, which refers in part to the tumor recapitulating pituitary embryology, the biologic behavior of pituitary blastoma is uncertain; about 50% of children with the disease succumb, with several deaths from early medical/surgical and later treatment-related complications. Metastasis has not been reported, and surgery leads to long-term survival in some children without additional oncologic therapies [22]. Hormonal replacement therapies are necessary.

9.4.2.3 Pineoblastoma

Pineoblastoma is a rare, aggressive embryonal tumor that arises in pineal gland. It usually occurs in childhood. It was first linked to *DICER1* in 2012 [95]; the association was confirmed 2 years later [96]. Recently, three publications extended these observations [97–99], showing that DICER1 and other miRNA biogenesis proteins are implicated in at least a quarter of all pineoblastomas. Methylation analysis of 70 pineoblastomas revealed 5 molecular sub-groups, and *DICER1* mutant pineoblastomas were exclusively placed in groups 1 and 2, which also included tumors with mutations in another miRNA processor, *DGCR8*, but excludes tumors with RB or MYC pathway alterations. Groups 1 and 2 were also quite distinct from the other three groups in terms of copy number profiles [97]. In this study, of the 23 cases that had germline *DICER1* testing, 5 (22%) had pathogenic variants. In a second study of 43 pineoblastomas, 12 (25%) were evaluated for germline *DICER1* pathogenic variants, and 3 cases were positive [98], and in the third, including 53 pineoblastomas, no cases had germline evaluation [99]. Overall, it appears that about one-fifth of pineoblastomas will occur as part of DICER1 syndrome and a third will have a germline pathogenic variant in *DICER1*, a somatic mutation in *DICER1*, or both. Notably, the second hit in DICER1-related pineoblastoma is much more likely to be a LOH event than in other *DICER1* mutant tumors—in a recent overview, 33/49 (67%) of *DICER1*-mutated pineoblastomas had LOH, compared with 26/619 (4%) in all other tumor types combined ($P < 0.0001$) [31]. This very high prevalence of complete loss of full-length DICER1 in pineoblastomas, together with mutually exclusive, frequent biallelic loss-of-function variants in other miRNA biogenesis genes in these tumors, suggests that the cell of origin of pineoblastoma is uniquely tolerant of miRNA perturbations [31]. Younger individuals with pineoblastoma are more likely to be associated with DICER1 syndrome than older individuals with pineoblastoma; pineoblastoma in a very young child may also result from other genetic causes such as *RB-1* alterations [97–99].

9.4.2.4 Primary CNS Sarcoma, DICER1-Mutant

Primary sarcomatous cerebral tumors are rare in children but are now increasingly recognized as a discrete phenotype in DICER1 syndrome. Like DICER1 anaplastic sarcoma of the kidney, these tumors have been described as PPB-like and are clearly DICER1-related sarcomas [3, 9, 14, 16, 96, 100–104]. Approximately 6 cases in DICER1-predisposed persons have been reported. The tumors revealed typical germline predisposing and/or somatic RNase IIIb *DICER1* alterations or occurred in children with other DICER1 phenotypes or phenotypic relatives [3, 9, 16, 31, 101, 104]; a total of 35 cases involving one or more germline and/or somatic *DICER1* alterations are documented [3, 9, 14, 16, 96, 100–104]. Among 13 cases where germline and tumor DNA have been studied, only 5 revealed germline involvement [31]. These data suggest that, in addition to DICER1 syndrome examples, exclusively somatic *DICER1* alterations contribute to many primary DICER1-related CNS sarcomas. Typical for most DICER1 syndrome tumors, primary CNS sarcoma—*DICER1* mutant tumors occur in the first two decades of life, with emphasis in the first 10 years. They predominantly affect the cerebral hemispheres; one has been reported in the brainstem, although it was not possible to distinguish it molecularly from possible metastasis of the patient's earlier DICER1-related sarcoma of the uterine cervix [96].

9.4.2.5 ETMR-Like Infantile Tumors

Eleven aggressive and unusual tumors resulting from *DICER1* alterations pathologically very similar to embryonal tumor with multilayered rosettes (ETMR) have been reported [43, 44, 100]. ETMR tumors encompass a set of aggressive early childhood CNS tumors that include entities previously described as ependymoblastoma and medulloepithelioma. They occur predominantly in children under 3 years of age [105]; 70% are supratentorial and 30% infratentorial [105]. Ninety percent of ETMR tumors reveal *C19MC* amplification, which is associated with dysregulated miRNA profiles [44]. About 10% of ETMRs do not demonstrate *C19MC* amplification ("*C19MC*-negative ETMR"), frequently have biallelic *DICER1* alterations ("ETMR *DICER1*-altered"), and tend to be infratentorial [105]. Thus, the *DICER1*-altered ETMR are likely to be infratentorial. ETMR tumors are highly likely to express LIN28A immunopositivity, regardless of their molecular signature [105]. Of the recently reported *DICER1*-altered ETMR, 11 of 11 had 2 *DICER1* alterations including an RNase IIIb variant; in 10 of the 11, the non-RNase IIIb variant was proven to be in the germline [43, 44, 100]. Three cases are reported in detail and occurred in infants less than 12 months of age; two were in the cerebellum [43, 100]; the third was large with an uncertain origin, but it was intraventricular and infiltrated the thalamus and superficially the cerebellar vermis [43, 100]. The three tumors were LIN28A positive; the pathology suggested ETMR, although in one child no true rosettes were identified [43]. None had *C19MC* amplification. The eight additional *DICER1*-altered ETMR tumors were reported with limited clinical

information; the site of the tumors was predominantly infratentorial [44]. An intracranial medulloepithelioma has been reported in one DICER1 syndrome kindred although molecular studies were not done [106].

In addition to *DICER1*-altered and *C19MC*-related ETMR tumors, two ETMR tumors have been reported with amplification of the *miR-17-92* miRNA cluster (*MIR17HG*) [44].

9.4.3 Ciliary Body Medulloepithelioma

Ciliary body medulloepithelioma (CBME) is a very rare embryonal ocular tumor occurring both sporadically and in association with DICER1 syndrome [107, 108]. CBME is histologically similar to medulloepithelioma within the ETMR tumors discussed above, and CBME is LIN28A positive [105]. However, CBME does not reveal *C19MC* amplification [105, 109]. As discussed below, *DICER1* alterations play a role in some CBME. Presenting with leukocoria and/or decreased visual acuity, CBME occurs in the latter half of the first decade of life in both DICER1-associated and presumed sporadic cases and usually leads to enucleation, but monitoring without surgery and in some cases with intraocular chemotherapy have been reported [110, 111]. CBME is classified histologically as teratoid or non-teratoid, either of which may be benign or malignant; cases associated with DICER1 syndrome are typically benign and a mixture of histologic types; one malignant CBME has been reported [9]. Bilateral disease has not been observed, although one individual with a mosaic predisposing *DICER1* alteration had multiple phenotypes as well as CBME in one globe; the other eye was "prephthisical secondary to anterior segment dysgenesis and had no light perception since early childhood" [7, 8, 112]. CBME cases are rare, and their incidence among carriers of pathogenic *DICER1* variants is difficult to ascertain. However, among approximately 300 PPB cases, 4 CBME were reported [110]; among 207 variant carriers studied systematically, 4 CBME were observed [19]; and among 103 variant carriers in a systematic survey who underwent comprehensive ophthalmologic examinations, 1 carrier had a history of CBME, and 2 carriers developed CBME 4.5 and 5 years, respectively, following normal comprehensive ophthalmologic examination [113]. These data involve ascertainment bias, and we estimate that 1% or less of variant carriers may develop CBME. A somatic RNase IIIb mutation has been reported in CBME and was not associated with a predisposing genetic abnormality [114]. A further six CBMEs harbored somatic RNase IIIb hotspot mutations; the germline status of these patients was not ascertained [9, 109, 115].

Congenital phthisis bulbi and related ocular dysplasia may also very rarely be related to *DICER1* mutation [8, 112, 116].

9.4.3.1 Nasal Chondromesenchymal Hamartoma

Nasal chondromesenchymal hamartoma (NCMH) is a very rare, benign tumor of the sinus and nasal cavities. Like some other tumors in DICER1 syndrome such as PPB and DICER1 anaplastic sarcoma of the kidney (discussed below), NCMH itself was first codified as a discrete pathologic entity in recent years [117]. Clinically, NCMH presents with nasal congestion and/or tissue at the nares. Histologically, NCMH is a complex mixture of cystic and solid cartilaginous and mesenchymal elements, typical of many DICER1 syndrome proliferations but without features of malignancy [117]. NCMH is an expansile proliferation in the nasal cavity and/or paranasal sinuses, may be bilateral, frequently effaces nearby delicate boney structures, and is managed with surgery even when there are recurrences [118, 119]. The co-occurrence of NCMH with PPB was noted in 2010 [118], and germline, mosaic, and somatic *DICER1* mutations have been demonstrated [119]. In the general population, NCMH occurs primarily in infants, whereas in DICER1 syndrome, NCMH is observed from the ages of approximately 6 to 21 years [117–119]. Its rarity is evidenced by 8 cases noted among 207 carriers of *DICER1* variants in DICER1 syndrome families [19]. Seven of the eight NCMH cases occurred in variant carriers who also had lung cysts and/or PPB. Other sinonasal proliferations than NCMH have also been observed in a small number of DICER1 syndrome patients and may result from coincidence [119].

9.5 Gastrointestinal Tract

9.5.1 Cystic Mesenchymal Hamartoma Liver

Apellaniz-Ruiz et al. reported on two children who developed hepatic cysts at very early ages (diagnosed at 26 months and 9 months, respectively) [45]. In the first child, hepatic resection of the partly cystic, partly solid lesion was followed by enlargement, and a repeat resection (hepatic lobectomy) was required 4 months later. The second child was found on magnetic resonance imaging at age 9 months to have a solid 14 mm liver tumor with clustered tiny cysts. The tumor gradually increased to 66 mm by age 39 months and had become predominantly cystic. At 75 months, a needle biopsy was undertaken. No resection was performed, and the cysts regressed over time. Subsequently the children developed other lesions typical for DICER1 syndrome, and both were found to possess germline pathogenic variants in *DICER1*. Only the first child's liver cyst gave a clear result—an RNase IIIb hotspot mutation was present. The authors decided, on the basis of the known lesions that occur in DICER1 syndrome, as well as the clinical presentation, course of the disease (including regression), pathological findings, and results of imaging studies, that the most plausible diagnosis was a cystic form of mesenchymal hamartoma of the liver (MHL) [120, 121]. This was strengthened by the known

association between *C19MC* and MHL and the pathological similarity between *DICER1*-related ETMR and *C19MC*-related ETMR (see above). However, two expert pathologists [122], on reviewing the published images, thought the correct diagnosis was solitary (nonparasitic) bile duct cysts of the liver [123]. Further studies will be required to resolve this controversy, but it seems that cystic hepatic tumors are a rare manifestation of DICER1 syndrome.

9.5.2 Hamartomatous Intestinal Polyps

Juvenile hamartomatous intestinal polyps have occurred in children in DICER1 syndrome kindred or in children with other DICER1 syndrome phenotypes; in some cases, constitutional *DICER1* mutations have been shown [124]. The most frequent site of the polyps is the ileum, where they may cause intussusception, but polyps from the esophagus to the rectum have been observed [4, 124]. Patients are typically under 5 years of age. The triad of intestinal polyps, lung cysts, and renal cysts in infants is strongly suggestive of DICER1 syndrome, perhaps especially so for *DICER1* RNase IIIb mosaicism [4, 7, 8, 32–35]. One published case of juvenile intestinal polyp had a proven predisposing *DICER1* alteration (a mosaic RNase IIIb variant) and a loss-of-function second mutation in a polyp [7]. In two cases, germline *DICER1* pathogenic variants were present, but polyp analysis revealed no somatic mutation or LOH [8, 45].

9.6 Gynecological Tract

9.6.1 Ovarian Sex Cord-Stromal Tumors and Other Ovarian Tumors

Ovarian sex cord-stromal tumors (OSCST), which include granulosa cell tumor and SLCT, are uncommon non-epithelial ovarian cancers definitively described by Young [125, 126]. As mentioned above, the association of ovarian SLCT (then known as arrhenoblastoma) and MNG was noted in 1974 [68]; germline pathogenic variants in *DICER1* were identified to be the genetic link (OMIM #138800) [71]. Both Schultz et al. and Slade et al. identified SLCT and other OSCST in PPB patients and their relatives in whom germline *DICER1* pathogenic variants were demonstrated [5, 127]. The pairing of SLCT with MNG or other hallmark DICER1 syndrome tumors in a proband or relatives is strongly suggestive of DICER1 syndrome [5, 71].

In 2011, Heravi-Mousavi et al. identified somatic *DICER1* RNase IIIb hotspot mutations in 60% of 43 unselected SLCT and initiated the search for such hotspot mutations in other phenotypes [25]. Subsequent studies have determined that

biallelic *DICER1* alterations characterize a large majority of SLCTs, particularly the moderately or poorly differentiated variants, including those with retiform areas and heterologous elements [27, 128–134]. Heterologous elements, including areas resembling rhabdomyosarcoma, may be a particular feature of some DICER1-related SLCT, but fully sarcomatous ovarian tumors are also seen in DICER1 syndrome, discussed below. At least 30% of reported moderately and poorly differentiated SLCTs arose in persons with germline *DICER1* mutations; it is likely that upward of 50% are syndromic [6, 27, 128–134]. It is speculated that the well-differentiated variant of SLCT is a fundamentally different tumor type from the moderate and poorly differentiated variants and is not DICER1 related [128]. Although most often unilateral, SLCT may occur bilaterally; bilateral SLCT is highly likely to be DICER1 related and, through genetic testing, has been found to represent independent primary tumors rather than metastatic neoplasms, which may have important implications for clinical management [135, 136].

SLCT associated with *DICER1* pathogenic variants can occur from early childhood to the fifth decade of life with most occurring from approximately age 10–25 years. Abdominal mass, abnormal menstruation, and hormonal perturbation such as androgenization are typical presenting signs. A sagittal section magnetic resonance image of DICER1-related left ovarian SLCT is shown in Fig. 9.5c.

Rarely, OSCST other than SLCT are DICER1 related, including juvenile granulosa cell tumor and pure Sertoli cell tumors [127]. Gynandroblastoma is an OSCST displaying both male and female sex cord differentiation; a subset of gynandroblastoma containing components of moderately or poorly differentiated SLCT and juvenile granulosa cell tumor exhibit *DICER1* hotspot mutations [137]. DICER1-related gynandroblastoma may represent morphological variants of SLCT [137].

Primary sarcoma of the ovary is another rare example of a DICER1-related sarcoma. Six cases have been reported: 5 were associated with germline *DICER1* pathogenic variants and bore somatic hotspot mutations [14, 127, 138–141] and one occurring in a 60-year-old harbored biallelic somatic *DICER1* mutations [13]. Other ovarian tumors, in particular the commonly seen epithelial ovarian tumors, are rarely if ever implicated in *DICER1* syndrome [25, 27, 142].

9.6.2 Embryonal Rhabdomyosarcoma of Uterine Cervix and Corpus

DICER1-related sarcoma of the uterine cervix is highly indicative of DICER1 syndrome. Known for years as cervical sarcoma botryoides or cervical embryonal rhabdomyosarcoma (ERMS), its association with SLCT has long been noted [126, 143–146]. It was definitively linked to *DICER1* alterations in 2011, and many cases have been reported [24, 26, 124, 147–149]. This disease presents in the second and third decades of life, most commonly among teenagers. Vaginal bleeding, passage of a vaginal polypoid mass, or a mass (often large) at the introitus are presenting

signs. The self-consciousness regarding sexual matters in teenage years probably accounts for delayed diagnosis in some cases. Recently it has been noted that cervical polyps judged benign, such as inflammatory polyps, can precede DICER1-related cervical sarcoma [150]; especially in any young woman with personal or family evidence of other syndrome phenotypes, molecular studies might reveal the *DICER1* involvement in polyps even when not frankly sarcomatous. In such instances, subtle benign-looking cervical polyps may in fact be sarcomas [151].

Two cases of DICER1 sarcomas of the uterine corpus have been reported in individuals with germline *DICER1* variants, and both tumors harbored an RNase IIIb hotspot mutation [152, 153]. Confusion as to the exact origin of some of these tumors is possible. In general, the cervical tumors are anatomically distinguishable from those arising from the uterine body; however, occasionally a tumor is considered to originate high in the vagina yet in fact may have a cervical origin.

9.6.3 Other Gynecologic Structures

A newly described entity which can involve various gynecologic structures is a "PPB-like" DICER1 sarcoma arising in the abdominal cavity from visceral or parietal peritoneum [13, 17]. Four such tumors appeared to arise from fallopian tube serosa. Biallelic *DICER1* mutations were identified in these tumors [13, 17]. The "PPB-like" moniker was used by one author because two of the tumors mimicked cystic Type I PPB [17]. A separate ERMS of the broad ligament bearing biallelic somatic *DICER1* alterations was fatal in a 23-year-old [154].

9.7 Kidney, Urinary Tract, and Testes

9.7.1 Cystic Nephroma, Anaplastic Renal Sarcoma, Wilms Tumor, and Bladder ERMS

Cystic nephroma (CN) and anaplastic sarcoma of the kidney are strongly associated with *DICER1* variants; Wilms tumor is very rarely encountered. CN is considered benign and is among the more frequent manifestations of DICER1 syndrome (Fig. 9.1, Table 9.1), occurring in between 5 and 10% of variant carriers [19, 35, 155, 156]. Except that CN cysts are fluid-filled, CN is grossly reminiscent of Type I PPB with exuberant clusters of thin-walled multilocular cysts (Fig. 9.5d). Microscopically CN also differs from Type I PPB in that there are no primitive cells. Using both molecular signatures and detailed pathologic examination, CN in young children can now be differentiated from a similar-appearing tumor in adult women (age > 50 years) with which it has been comingled in the past [157, 158]. Like PPB, CN may be bilateral and occurs in early childhood as most pathological diagnoses

are made by age 48 months [35, 156, 159]; radiographic detection may occur later [19, 160]. CN may develop after a normal ultrasound, and one unusual case revealed CN developing at age 12 years in a child followed closely with frequent abdominal imaging because of an earlier ovarian tumor [138]. Although classified pathologically as non-neoplastic, CN can progressively efface renal parenchyma leading rarely to bilateral nephrectomy and renal replacement [34]. Segmental or complete nephrectomy is frequent. Stable multicystic lesions or small areas of "recurrence" following partial nephrectomy have been untreated without complication, and because CN may be detected incidentally beyond early childhood, resecting CN may not be essential although more data must be collected [7]. Childhood CN is strongly associated with *DICER1* mutations: among 20 unselected cases, 15 had germline *DICER1* mutations, and 18 had somatic RNase IIIb hotspot mutations [156]. Bilateral CN and familial CN are highly suggestive of *DICER1* involvement [35, 155].

Cystic partially differentiated nephroblastoma (CPDN) is a cystic neoplasm with similarities to CN and to cystic Wilms tumor [158]. The few cases of CPDN studied have not revealed *DICER1* variants, and it has not been reported in DICER1 syndrome [156].

In 2007, anaplastic renal sarcoma was described as a distinct new pathologic entity among 20 individuals ranging in age from 10 months to 41 years, median age 5 years [49]. The authors noted that the tumors had the appearance of PPB and seven cases had distinct cystic components; as described earlier, this disease is a characteristic DICER1 sarcoma (Table 9.1). Between 2014 and 2018, several reports described additional cases of anaplastic renal sarcoma and an association with somatic and/or classic biallelic *DICER1* alterations and/or clinical evidence of DICER1 syndrome. These cases have led to the label "DICER1 anaplastic sarcoma of kidney" (D1ASK). In addition, some D1ASK are associated with prior or concurrent diagnoses of CN [136, 156, 161]. In one complex case, a cystic renal tumor appeared to be CPDN except that it manifested scattered anaplastic nuclei and atypical mitoses not consistent with CPDN; after germline and somatic *DICER1* mutations were identified, the tumor was finally considered to be an incipient D1ASK, i.e., CN in transition to D1ASK [161]. The association of prior CN with later D1ASK may mimic transition of Type I PPB into Type II or III PPB, although CN→D1ASK appears much less frequent than pulmonary progression [156]. That D1ASK may follow CN raises the question of whether all remnants of CN should be surgically extirpated; because the phenomenon is so infrequent, firm recommendations about resecting a clinically asymptomatic CN cannot yet be made.

Wilms tumor has been reported in several families with DICER1 syndrome and in individuals with *DICER1* alterations making *DICER1* a rare cause of this tumor [5, 7, 20, 28, 37, 96, 124, 153, 162–165]. However, large surveys of the genetics of Wilms tumor reveal that it is only rarely associated with predisposing *DICER1* alterations, at most in 1% of cases [166–170]. Expert pathology review is important because D1ASK can be misinterpreted as Wilms tumor [136]. Of narrow importance are the observations of three Wilms tumors associated with the *DICER1* RNase IIIa mutation c.4031C>T, p.Ser1344Leu; two mutations were somatic in

tumor [28, 166] and one was in the germline [37]. There were additional pathologies in all three children which led to genetic testing. Also, a study of 48 families with multiple Wilms tumors found 2 families with distinct *DICER1* alterations [122]. Except for the above rare examples, a sporadic case of Wilms tumor should not raise suspicion for *DICER1* involvement unless other DICER1 phenotypes appear in the patient or their family.

Despite the lack of a frequent connection between predisposing *DICER1* alterations and Wilms tumor, exclusively somatic mutations in the broad family of miRNA processing genes (*DROSHA*, *DGCR8*, *DICER1*, *XPO5*, and *TARBP2*) indeed play a role in up to one-third of Wilms tumors [1, 166–170].

9.7.2 Bladder DICER1 Sarcoma (and Other Childhood Rhabdomyosarcomas)

DICER1 sarcoma in the urinary bladder has been reported in several children in DICER1 syndrome kindred or children who harbor germline *DICER1* alterations [4, 24, 106, 136, 171]. In one case, biallelic mutations have been demonstrated [136]. The children have been diagnosed from the first though the 14th years of life; both males and females are affected.

Bladder ERMS is among the sites of classic early childhood ERMS, which include also the vulva and vagina, the paratesticular tissues, the prostate, and the orbit/nasopharynx/parameningeal site. Among these sites, only for bladder ERMS have multiple examples been associated with DICER1 syndrome. A vaginal ERMS is reported at age 5 years [160]. An unusual low-grade myxoid sarcoma in the paratestis is reported in a child with a germline *DICER1* variant who also had CN (Table 9.1); both tumors harbored an RNase IIIb hotspot mutation [172]. The same publication reports no *DICER1* alterations in 13 paratesticular ERMS [172]. To our knowledge, surveys for *DICER1* alterations of ERMS in the other typical childhood sites have not been reported.

9.7.3 Testicular Tumors

Germline pathogenic variants in *DICER1* do not appear to predispose to testicular germ cell tumors [142, 173]. The equivalent testicular tumors to ovarian Sertoli-Leydig cell tumor would be Sertoli cell tumors and Leydig cell tumors. There is an unpublished report of a young boy with a germline *DICER1* pathogenic variant who developed a PPB and Sertoli cell tumor of the testis that contains an RNase IIIb hotspot mutation, but clearly, they are extremely rare occurrences.

9.8 Other Abdominal Tumors

9.8.1 Presacral Malignant Teratoid Tumor

Two very young infants have been recently reported with unusual DICER1-related tumors labeled presacral malignant teratoid tumors (Fig. 9.1, Table 9.1) [160]. A 1-week-old child had an intraspinal-canal extradural tumor from vertebral body L2 to the sacrum. A 4-month-old child had a large presacral pelvic mass. Pathologically the first tumor was multipatterned with a mixture of medulloepithelioma-like and mesenchymal ERMS elements. The second tumor was composed of primitive neuroepithelium and ERMS elements. Both tumors had immature cartilage, and neither had broader germ layer components to be considered malignant teratomas. Both tumors harbored a germline loss-of-function *DICER1* pathogenic variant coupled with a typical somatic RNase IIIb hotspot mutation. The first child succumbed to recurrent disease after a few months. The second child survived and later developed vaginal ERMS, D1ASK in a CN, papillary thyroid microcarcinoma, CN in the contralateral kidney, and NCMH.

9.8.2 PPB-Like Peritoneal Sarcoma

Discussed briefly above with gynecologic tumors are two recent reports describing several children and one adult with "PPB-like" DICER1 sarcomas arising in the abdominal cavity from visceral or parietal peritoneum; two of the cases appear to be included in each report [13, 17]. The patients ranged from 3 to 14 years of age (median age 13 years) with one additional primary ovarian sarcoma in a 60-year-old woman; all but one of the cases were in females. With the exception of the ovarian sarcoma [13], the tumors arose upon the serosal surfaces of fallopian tubes ($n = 4$), colon ($n = 1$), and pelvic sidewall ($n = 2$). Two of the tumors comprised clusters of cysts resembling Type I and Ir PPB, respectively, leading Schultz et al. to identify their cases as "PPB-like peritoneal sarcomas" mimicking the cystic to solid continuum of PPB [17]. Apart from the cystic tumors, the tumors were complex DICER1 sarcomas. In six of the seven cases, tumor DNA revealed biallelic loss-of-function and RNase IIIb *DICER1* alterations. Four of five tested children had germline *DICER1* alterations. Six of the seven children survived 10–155 months (median 65 months) from peritoneal sarcoma diagnosis.

9.9 Rare or Possible Associations

DICER1 syndrome features the characteristic tumors and dysplasias discussed above, but in addition, children with DICER1 phenotypes or their kindred may develop other pediatric tumors. Although some such tumors may be coincidental, in

view of the pleiotropy of the syndrome, some can result from DICER1 dysfunction, particularly if similar tumors have been observed in syndromic settings. For example, sarcomas with pathological features other than the typical DICER1-related sarcoma [48] have been reported in persons with germline *DICER1* mutations (synovial sarcoma in in a cousin of a PPB patient [3], pleomorphic sarcoma with leiomyosarcomatous features in a woman aged 26 years [124], and paraspinal rhabdomyosarcoma at age 20 years [71] (see also the low-grade myxoid paratesticular sarcoma discussed below [172])).

Pulmonary sequestration was discovered in a child with a germline *DICER1* mutation and CN [174]; the sequestration specimen was found not to have a typical RNase IIIb mutation (de Kock and Foulkes, unpublished data). Two other children with pulmonary sequestration were found to harbor PPBs within resected sequestration specimens (Types I and II, respectively) [175, 176]. In each case, the sequestrations had non-pulmonary artery feeding vessels and had pleural membranes distinct from nearby lobes. In neither of these children were molecular studies performed. Transposition of the great arteries was noted in a *DICER1*-affected kindred [124], but a larger study of transposition of the great arteries suggested that there is no association with germline *DICER1* variants [177]. Three cases of Ewing sarcoma-type tumors have been observed: one arising in the cervix [124] and two on the chest wall ([154] and de Kock and Foulkes, unpublished). In the former case, the patient carried a germline *DICER1* pathogenic variant, but the cervical tumor was not evaluated; in the latter two cases, the germline revealed a loss-of-function *DICER1* pathogenic variant, but there was no "second hit" in *DICER1* in the tumors ([154] and de Kock and Foulkes, unpublished). A child reported to have CCAM (later considered to be Type I PPB) also had an intracranial vein of Galen cyst; no molecular studies were done [178].

Various other classical childhood tumors may arise incidentally in *DICER1* variant carriers. For example, neuroblastoma has been noted, but no study has identified a somatic *DICER1* mutation to indicate canonical *DICER1* causality [5, 179–181]. An atypical choroid plexus papilloma in a child with PPB Type I was carefully determined not to be related to *DICER1* mutation [38, 182]. In contrast, the recent report of one male with an unusual low-grade myxoid paratesticular sarcoma is probably a true, albeit very rare, association, in view of the presence of a germline *DICER1* pathogenic variant, his history of CN, and the presence of different somatic RNase IIIb missense hotspot mutations in the sarcoma and the CN [172]. Similarly, the sole report of a well-differentiated fetal lung adenocarcinoma arising in a child with DICER1 syndrome is supported by the presence of a characteristic "second hit" in the tumor [183]. There is a single unpublished report of a child with a germline *DICER1* pathogenic variant, PPB, and a testicular Sertoli cell tumor, wherein the testicular tumor has a typical RNase IIIb hotspot mutation, making it likely that this is a true association. Other very rare but real associations are likely to continue to emerge.

Adult-onset tumors have been reported in DICER1 syndrome, but these could be incidental. For example, Cotton and Ray reported on the case of a pituitary microadenoma (prolactinoma) occurring in a 50-year-old woman with DICER1

syndrome, but no molecular work was performed on the pituitary tumor [184]. The patient may have had the prolactinoma for over 20 years, but early-onset pituitary adenomas are not known to be related to DICER1 syndrome [185]. A pathologically diagnosed prolactinoma occurred in a 25-year-old woman who also had SLCT and was in a DICER1 kindred; the tumor was not studied [JRP personal observation]. In another DICER1 kindred with a proven *DICER1* germline variant, at age 43 years, the brother of a man (with childhood lung cysts and an eye tumor) had a radiographically diagnosed pituitary microadenoma which was treated chemically [JRP personal observation]. Prolactinomas are not rare, but with pituitary blastoma such a characteristic DICER1 tumor, these observations of prolactinoma deserve attention.

9.10 Non-neoplastic Phenotypes in DICER1 Syndrome

Four surveys have systematically studied various anatomical sites of *DICER1* variant carriers and identified certain non-neoplastic manifestations of DICER1 syndrome. The studies focused on auxology, kidneys and urinary tract, eyes, and dentition.

In the auxology study, various body measurements were compared between 76 known *DICER1* variant carriers and 53 *DICER1* wild-type family members [186]. Both male and female variant carriers had significantly larger occipito-frontal head circumference (OFC) than population norms and family controls ($p < 0.001$); 42% of all carriers (33% of males and 50% of females) were "macrocephalic" defined as OFC greater than the 97th percentile of published norms; however, the difference between carriers and controls was only significant among adults. In general, the OFCs of variant carriers ranged from the 50th to above the 97th percentiles of published norms, whereas OFCs of family controls ranged between the 3rd and 97th percentiles. Variant carriers were significantly taller than family controls ($p = 0.048$), but the proportions of both variant carriers and family members above the 97th percentile for height were similar and not different from population controls. Large OFC did not correlate with height. There were no differences between carriers and family controls in upper body length (symphysis pubis to top of head) versus lower body length (symphysis pubis to floor) nor in arm span.

In the study of kidney and urinary collecting system structures, 89 *DICER1* variant carriers were compared to 61 *DICER1* wild-type family controls using renal ultrasound and blood and urine biochemical tests [159]. A renal cyst was detected in 1 of 33 children who did not have a prior history of CN (8 of 41 carrier children had had a prior CN diagnosis). In adults, ultrasound-detected renal cysts were similar in carriers and controls (in ~20% of each group). Eight of 89 variant carriers had ultrasound-detected kidney and collecting system anomalies, which included nephrocalcinosis, nephrolithiasis, and structural abnormalities of varying severity. The structural anomalies involved partially duplicated collecting system ($n = 2$), collecting system dilatation following a uretero-pelvic junction repair ($n = 1$) and

incomplete rotation of the kidney ($n = 1$). There were no notable biochemical differences between study subjects based on plasma, serum, or urine chemistries.

In the ophthalmologic study, 103 *DICER1* variant carriers were compared to 69 *DICER1* wild-type family controls using a wide array of detailed ophthalmologic examinations (which did not include imaging modalities) [113]. Among variant carriers, 97% had visual acuity of 20/40 or greater; 23 variant carriers (22%) had various ocular abnormalities of retinal pigment, increased cup-to-disc ratio, or optic nerves compared to 4 such findings in controls ($p = 0.005$). One carrier had the unexpected finding of retinitis pigmentosa with a novel variant of unknown significance in *PRPF31*. Three of the 103 variant carriers had developed CBME by the time the study was reported: one prior to enrollment in the study and two 7-year-old carriers developed CBME 4.5 and 5 years, respectively, following normal findings in the extensive evaluations of the study. Each of these two children had developed symptomatology (vision loss and strabismus, respectively) which led to ophthalmologic examination and discovery of CBME.

In the study of the dental phenotype of DICER1 syndrome, 57 *DICER1* variant carriers were compared to 55 *DICER1* wild-type family controls [187]. Each was evaluated with dental examination, dental radiographs, and oral photograph. Compared to family controls, carriers were significantly more likely to exhibit periodontitis and bulbous crowns. A bulbous crown is a relative ballooning of the crown of the tooth and relative belt-like constriction at the junction between the crown and root, whereas a normal tooth has a less full crown and a gradual tapered profile as the crown blends to the root. In the molars of variant carriers, the examiners also noted taurodontism in which the upper body of the tooth (and its internal pulp cavity) is relatively enlarged compared to the roots, with the result that the roots are correspondingly shorter than normal. In logistic regression analysis, only bulbous crowns and periodontitis were confirmed as significant observations in carriers versus family controls.

9.11 Notes on Tumor Surveillance in DICER1 Syndrome

Although the DICER1 phenotype will likely evolve, recognition and genetic confirmation of the syndrome are now practical. Commercial and research *DICER1* assays are available from several sources. When routine studies fail to identify a pathogenic *DICER1* variant, highly suggestive cases may be solved by more intensive analysis including evaluating for large or small deletions or mosaicism [188]. A diagnosis of even one of the highly characteristic DICER1 phenotypes deserves suspicion (Fig. 9.1, Table 9.1). Two or more of the less distinctive conditions in a patient or family also deserve inquiry. Because of low penetrance and pleiotropy, detailed family histories may reveal unexpected associations [106], and focused pathologic reevaluations may reveal associated diagnoses.

For affected kindred and their caregivers, questions of carrier identification, genetic counseling, disease screening, and early diagnosis will arise. In general,

the authors recommend genetic counseling, testing for carrier status, and continuing family education. That many carriers are unaffected and diseases often not life-threatening may reassure families (Table 9.1). Once variant carriers are identified, screening for DICER1 phenotypes must be addressed; many factors influence the reasonableness of screening. Is the goal of screening early detection and will early detection likely affect outcome? Will screening provide reassurance to families or cause excess anxiety? Penetrance is generally quite low, and several conditions are exceedingly rare. Phenotypes like MNG and ovarian stromal tumors present along a protracted age spectrum from approximately 5 years or earlier to 40 years and beyond. In contrast, PPB, CN, pituitary blastoma, the ETMR-like cerebral tumors, CBME, and NCMH occur in narrower age ranges (Table 9.1). Family education should include the differences among phenotypes and also include some warnings like respiratory distress or "pneumonia" as possible signs of PPB and menstrual changes or hirsutism as signs of a cervical or ovarian tumor, respectively. Screening modalities can include questioning for symptoms or signs, physical examinations, and imaging which may range from less invasive ultrasound to computed tomography or magnetic resonance, which in infants may require anesthesia. The frequency of screening must address the conflict between high frequency, which might actually result in early detection, or low frequency with reduced chances that a disease will be detected before it becomes symptomatic.

Screening for ovarian stromal cell tumors exemplifies the problem: the age range for diagnosis extends from age ~2 years to age 40–50 years or more. Death from SLCT and other ovarian phenotypes appears to be uncommon. Do these circumstances support years of screenings such as abdominal or vaginal ultrasound? And at what intervals? In contrast, PPB occurs in a much tighter age range from birth to age 72 months. Whether serial chest radiographs or computed tomography with its higher likelihood of detection is justified will likely be local decisions with family input. And again, how often? The authors suggest that careful education as well as reasonable efforts at reassurance can help DICER1 families, with recognition that each family will have its own determinants for reassurance, which are likely to evolve over time.

There have been some expert recommendations about screening, but they vary widely. At one institution, frequent evaluations including annual whole-body magnetic resonance imaging are suggested [189]. A broad consortium of physicians has published screening guidelines that address each phenotype and organ system [56]. The most focused screening schema involves the detection of cystic PPB in proven *DICER1* variant carriers in the first 2 years of life and is based on the observation that extirpation of cystic Type I PPB may prevent evolution of such a lesion to aggressive Types II or III PPB [55].

9.12 Late-Breaking Update, April 2021

9.12.1 DICER1 Sarcomas

In a similar fashion to this opening section, McCluggage and Foulkes [192] have put forward the notion that many of the diagnostically discrete entities that are discussed in Sects. 9.2, 9.6.2, 9.6.3, 9.7.1, 9.7.2, 9.8.1, and 9.8.2 share common features and have called for efforts to unify the nomenclature. In support of this, Kommoss et al. [193] have recently reported that DICER1-related genitourinary ERMS share distinct methylation profiles that distinguish them from non-DICER1-related ERMS. This paper is also relevant to Sect. 9.6.2.

9.12.2 Chest

Some PPBs, especially later-stage tumors, harbor somatic *TP53* mutations. When present, the PPBs appear to have a worse outcome. This may be important in management [194].

9.12.3 Cranial and Intracranial Tumors

Two important papers on pituitary blastoma have described the clinicopathological and molecular features, respectively—the former showing that nearly half of the children have died, and that resection extent was associated without outcome [195]; the latter showed that PRAME, an antigen expressed in normal testes, is significantly over-expressed in pituitary blastoma [196].

9.12.4 Gastrointestinal Tract

A recent case report described a primary biphasic hepatic sarcoma with an RNase IIIb somatic mutation in a child with DICER1 syndrome [197]. The authors of the report consider this entity to be the sarcomatous equivalent of the lesion described by Apellaniz-Ruiz et al., reported in Sect 9.5.1.

9.12.5 Kidney, Urinary Tract, and Testes

Mentioned in Sect. 9.9, the first report of a Testicular Stromal Cell tumor with both germline and somatic hotspot alterations has emerged. Clearly the testes are much more rarely affected than the ovaries, but it is likely that additional reports, possibly also of Leydig cell tumors, will be identified in future [198].

9.12.6 Rare or Possible Associations

The child described in [37] has subsequently been determined to have had a well-differentiated fetal lung adenocarcinoma (with a second *DICER1* "hit"), supporting the finding first reported in [183]. The question of whether Ewing's sarcoma is part of DICER1 syndrome was posed in this section, as no Ewing's tumor occurring in person with DICER1 syndrome had been found to have a hotspot second hit. The story has become more complicated as there is a recent case report of a 16-year-old female who was diagnosed with a high-grade undifferentiated cancer consisting of blastemal-like small blue cells [199]. The *EWSR1* gene rearrangement was found in ~16% of nuclei, but no fusion partner was found. A somatic hotspot mutation in *DICER1* was identified, however. Thus, it remains uncertain whether classical Ewing's tumor is part of DICER1 syndrome.

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Chapter 10
Cancer-Prone Inherited Bone Marrow Failure, Myelodysplastic, and Acute Myeloid Leukemia Syndromes

Sharon A. Savage, Lisa J. McReynolds, Marena R. Niewisch, Burak Altintas, D. Matthew Gianferante, and Blanche P. Alter

Abstract The inherited bone marrow failure syndromes (IBMFS) are a clinically and molecularly heterogeneous group of cancer-prone disorders. Many IBMFS are associated with specific clinical characteristics and/or dysmorphic features. The IBMFS associated with increased risk of leukemia, myelodysplastic syndrome (MDS), and certain solid tumors include Fanconi anemia, dyskeratosis congenita, and Diamond Blackfan anemia. Individuals with Shwachman Diamond syndrome, severe congenital neutropenia, congenital amegakaryocytic thrombocytopenia, or thrombocytopenia absent radius syndrome may be at increased risk of leukemia. Patients with IBMFS have syndrome-specific pathogenic germline variants in genes critical in DNA repair, telomere biology, ribosome biology, or hematopoiesis.

There is a growing recognition of inherited forms of MDS and/or acute myeloid leukemia (AML) in people without classic IBMFS features. Many such patients have pathogenic germline variants in genes encoding transcription factors critical in hematopoiesis or cellular signaling. These individuals sometimes have a family history of MDS and/or AML and may or may not have other clinical features.

The diagnosis of cancer-prone IBMFS and MDS/AML syndromes is often challenging due to phenotypic overlap and variable clinical presentations. This chapter will review the clinical features, diagnosis, management, genetics, and pathophysiology of the cancer-prone syndromes associated with hematopoietic defects.

Keywords Fanconi anemia · Dyskeratosis congenita · Diamond Blackfan anemia · Shwachman Diamond syndrome · Thrombocytopenia absent radius syndrome · Congenital amegakaryocytic thrombocytopenia · Severe congenital neutropenia · Familial myelodysplastic syndrome · Inherited bone marrow failure syndromes ·

S. A. Savage (✉) · L. J. McReynolds · M. R. Niewisch · B. Altintas
D. M. Gianferante · B. P. Alter
Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA
e-mail: savagesh@mail.nih.gov

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10.1 Introduction

Early-onset bone marrow failure (BMF) is the primary clinical problem in the inherited bone marrow failure syndromes (IBMFS). However, many of the IBMFS are also associated with an elevated risk of myelodysplastic syndrome (MDS), leukemia (usually acute myeloid leukemia, AML), and specific solid tumors. The more common of the rare cancer-prone IBMFS include Fanconi anemia (FA), dyskeratosis congenita (DC), Diamond Blackfan anemia (DBA), Shwachman Diamond syndrome (SDS), and severe congenital neutropenia (SCN). Studies of families in which more than one person has MDS and/or acute myeloid leukemia have often led to the identification of these and other inherited MDS/AML disorders.

Patients with an IBMFS may have dysmorphic features or clinical findings associated with the disorder. However, a single lineage cytopenia may be the only presenting sign of any of these disorders. An inherited disorder should be considered in all patients presenting with BMF, regardless of the degree of failure or the involved lineages. Understanding of pathophysiology and clinical complications of these disorders is growing daily, but these disorders can still be diagnostic dilemmas. They may be under-recognized in both pediatric and adult hematology/oncology practices. Proper diagnosis is crucial for patient management and counseling of the entire family.

10.2 Fanconi Anemia (FA)

10.2.1 Clinical Features of FA

FA is a chromosome instability disorder caused by defective DNA repair. The clinical features include specific congenital anomalies (Fig. 10.1), progressive pancytopenia, and cancer susceptibility [1, 2]. Radial bone and thumb abnormalities, short stature, skin pigmentary changes (e.g., café-au-lait macules), renal malformations, and microcephaly are the most commonly reported features. FA is a phenotypically heterogeneous disease, and defects in multiple other organ systems have been reported including facial, skeletal, ocular, aural, genital, gastrointestinal, cardiac, and nervous system [2]. It is important to note that 5–30% of patients with FA are reported to have VACTERL-H association (presence of at least three of eight features: vertebral anomalies, anal atresia, cardiac anomalies, tracheo-esophageal fistula, esophageal atresia, renal structural anomalies, upper limb anomalies, and

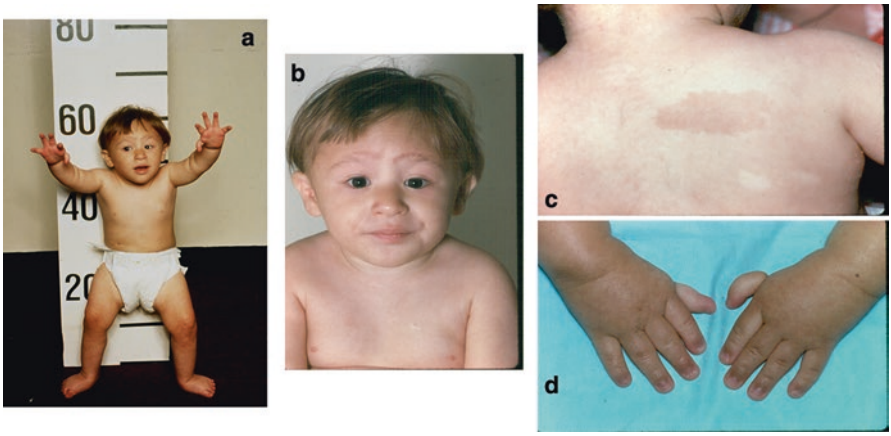

Fig. 10.1 Characteristic clinical features of Fanconi anemia. Features include short stature (**a**), microcephaly, dangling thumbs (**a**, **d**), characteristic facies (**a**, **b**—epicanthal folds, microphthalmia, triangular face), café-au-lait macules, and hypo-pigmented areas of the skin (**c**). He also has dislocated hips, which prevent him from standing straight, and rocker bottom feet (**a**). Surgery for imperforate anus and ureter reimplantation were required. (Previously published in Alter BP. Inherited bone marrow failure syndromes. In: Nathan DG, Orkin SH, Ginsburg D, Look AT, editors. Nathan and Oski's hematology of infancy and childhood, Vol. 1. Philadelphia: W.B. Saunders; 2003. p. 280–365)

hydrocephalus), and this is frequently associated with pathogenic variants in *FANCB*, *FANCD1*/*BRCA2*, *FANCD2*, or *FANCI* [2–6]. Recognizing these features is required to facilitate early diagnosis and surveillance for complications of FA. Other manifestations of FA include endocrine and metabolism problems (hypothyroidism, diabetes/glucose intolerance, dyslipidemia, pituitary abnormalities, growth problems, early menopause, and infertility), hearing abnormalities, and cataracts [7]. Despite the relative high rate of congenital malformations in FA, it is very important to recognize that 20–40% of patients with FA do not have these features [2]. BMF in FA generally occurs within the first decade of life. It may manifest as a mild or moderate single, bilineage cytopenia or pancytopenia with red blood cell (RBC) macrocytosis and elevated levels of fetal hemoglobin (HbF) [8]. Over time, the BMF may progress to the point where medical intervention is required. Early-onset BMF or head and neck or anogenital squamous cell carcinoma (SCC) may be the first presenting sign of FA.

10.2.2 Diagnosis of FA

The diagnosis of FA is often made based on congenital anomalies (e.g., absent thumbs) and early-onset BMF (Fig. 10.1, Table 10.1). A family history of FA, suggestive of cancer predisposition, or severe toxicity after radiation or chemotherapy may be informative but is rarely present. The median age at diagnosis is generally

Table 10.1 Features of the IBMFS with increased cancer risk. All disorders may present with or without family history or as a result of de novo mutations in the proband. The clinical features and diagnostic modalities are described in the text. Patients with these disorders may present with some, but not necessarily all, of the features listed. Clinically silent carriers are possible

Disorder	Clinical features	Laboratory findings	Biological pathway	Inheritance: known genes	Associated cancers
Congenital amegakaryocytic thrombocytopenia	Petechiae or hemorrhage in infancy	Reduced megakaryocytes in infancy	TPO receptor defects	**AR:** *MPL*	MDS, AML
Diamond Blackfan anemia	Severe anemia, typically in infancy, ~25% with birth defects	Elevated RBC ADA, macrocytosis, elevated HbF	Ribosome biogenesis	**AD:** *RPS7*, *RPS10*, *RPS15A*, *RPS17*, *RPS19*, *RPS24*, *RPS26*, *RPS27*, *RPS28*, *RPS29*; *RPL5*, *RPL11*, *RPL15*, *RPL18*, *RPL26*, *RPL27*, *RPL31*, *RPL35*, *RPL35A* **XLR:** *GATA1*, *TSR2*	MDS, AML, osteosarcoma, colorectal adenocarcinoma, and genitourinary cancers
Dyskeratosis congenita	Nail dysplasia, abnormal skin pigmentation, oral leukoplakia; BMF, pulmonary fibrosis, pulmonary arteriovenous malformations, stenosis of esophagus, lacrimal ducts, or urethra, liver disease, gastrointestinal bleeding	Very short telomeres, macrocytosis, elevated HbF	Telomere biology	**XLR:** *DKC1* **AD:** *TERT*, *TERC*, *TINF2*, *RTEL1*, *NAF1*, *PARN*, *ACD* **AR***: NOP10*, *NHP2*, *WRAP53*, *RTEL1*, *TERT*, *CTC1*, *STN1*, *POT1*, *PARN*, *ACD*	MDS, AML, head and neck squamous cell cancers

Fanconi anemia	Radial ray anomalies, short stature, microcephaly, café-au-lait spots, structural renal anomalies, BMF	Increased chromosome breakage in clastogenic assay, macrocytosis, elevated HbF	DNA repair	**XLR:** *FANCB* **AD:** *FANCR(RAD51)* **AR:** *FANCA*, *FANCC*, *FANCD1(BRCA2)*, *FANCD2*, *FANCE*, *FANCF*, *FANCG(XRCC9)*, *FANCI*, *FANCJ(BRIP1)*, *FANCL*, *FANCM*, *FANCN(PALB2)*, *FANCO(RAD51C)* *FANCP(SLX4)*, *FANCQ(ERCC4)*, *FANCS(BRCA1)*, *FANCT(UBE2T)*, *FANCU(XRCC2)*, *FANCV(MAD2L2)*, *FANCW(RFWD3)*	Squamous cell cancers of the head, neck, and anogenital region; neuroblastoma, Wilms tumor, medulloblastoma, MDS, AML
Shwachman-Diamond syndrome	Pancreatic insufficiency, short stature, metaphyseal dysostosis, neutropenia	Low serum isoamylase and trypsinogen, neutropenia	Ribosomal maturation, mitotic spindle stabilization	**AR:** *SBDS*, *DNAJC21*, *EFL1* **AD:** *SRP54*	BMF, MDS, AML, ALL
Severe congenital neutropenia	Frequent bacterial infections	Profound neutropenia; maturation arrest at promyelocyte/ myelocyte stage	Neutrophil function defects	**AD:** *ELANE (ELA2)*, *GFI1*, *G6PC3* **AR:** *HAX1* **XLR:** *WAS*	MDS, AML
Thrombocytopenia absent radii	Absent radii with thumbs present, petechiae or hemorrhage in infancy	Thrombocytopenia in infancy/ childhood, usually improves with time	Not yet elucidated	**AR:** 1q21.1 deletion and *RBM8A* variant	AML, ALL

Abbreviations: *TPO* thrombopoietin; *MDS* myelodysplastic syndrome; *AML* acute myeloid leukemia; *ALL* acute lymphocytic leukemia; *AD* autosomal dominant; *AR* autosomal recessive; *XLR* X-linked recessive; *RBC* red blood cell; *ADA* adenosine deaminase; *HbF* fetal hemoglobin; *BMF* bone marrow failure

between 7 and 9 years of age, with 75% of cases diagnosed between the ages of 4 and 14; however, FA has been diagnosed in neonates as well as in adults in their 50s or older. The FA diagnostic test is based on the detection of increased chromosomal breakage in cells cultured with a clastogen [9, 10]. The test is usually performed on fresh T-lymphocytes cultured with mitomycin C or diepoxybutane (MMC or DEB). Chromosome breaks and radial figures are quantified and compared with controls. If there is a high suspicion of FA but an equivocal lymphocyte chromosome breakage test result, chromosome breakage should also be tested on cultured skin fibroblasts. Hematopoietic somatic mosaicism is diagnosed if the lymphocyte chromosome breakage rate of breaks/cell, or the percent of cells with breaks, is normal, but the fibroblast values are in the FA range. A patient with mosaicism may occasionally be suspected if there are a small number of lymphocytes with a large number of breaks or radial figures; fibroblast cultures are used for confirmation [11]. Genetic diagnosis of FA can be established by single-gene testing, multigene panels, and whole-exome or whole-genome sequencing. Testing for deletions should also be performed if only one pathogenic variant is identified. Identification of causative variants helps confirm the FA diagnosis and changes the direction of genetic counseling.

10.2.3 Genetics and Pathophysiology of FA

All FA subtypes (formerly called "complementation groups") are inherited in an autosomal recessive (AR) pattern [12] with the exception of group B, which is X-linked recessive [13], and group R, which is autosomal dominant [14]. FA is caused by germline mutations in key components of the DNA repair pathway (Table 10.1). To date, there are 22 known FA subtypes (A, B, C, D1 (*BRCA2*), D2, E, F, G, I, J (*BRIP1*, *BACH1*), L, M, N (*PALB2*), O (*RAD51C*), P (*SLX4*), Q (*ERCC4*), R (*RAD51*), S (*BRCA1*), T (*UBE2T*), U (*XRCC2*), V (*MAD2L2*, *REV7*), W (*RFWD3*)) [12, 15, 16]. In most instances of AR FA, each parent has a single copy of a mutated gene, and the patient may be homozygous or biallelic for mutations in that gene. The heterozygote carrier frequency of FA was estimated to be 1 in 181 in the United States, and it may be higher in populations with founder effects, e.g., 1 in 90, such as Ashkenazi Jewish *FANCC* [17] as well as Afrikaners and sub-Saharan Blacks [18, 19].

The FA genes encode proteins that work in a coordinated manner to resolve DNA interstrand cross-links during cellular replication by the actions of nucleotide excision repair and homologous recombination (Fig. 10.2). Several excellent recent reviews on the FA proteins have been published [12, 20–24]. In brief, the FA protein core complex is a large nuclear E3 ubiquitin ligase complex consisting of FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, FANCM, and FANCT/UBE2T. The core complex interacts with FANCD2 and FANCI to form the "ID complex," which then interacts with FANCD1/BRCA2, FANCN/PALB2, FANCJ/BRIP1/BACH1, FANCP/SLX4, FANCO/RAD51C, FANCQ/ERCC4/XPF,

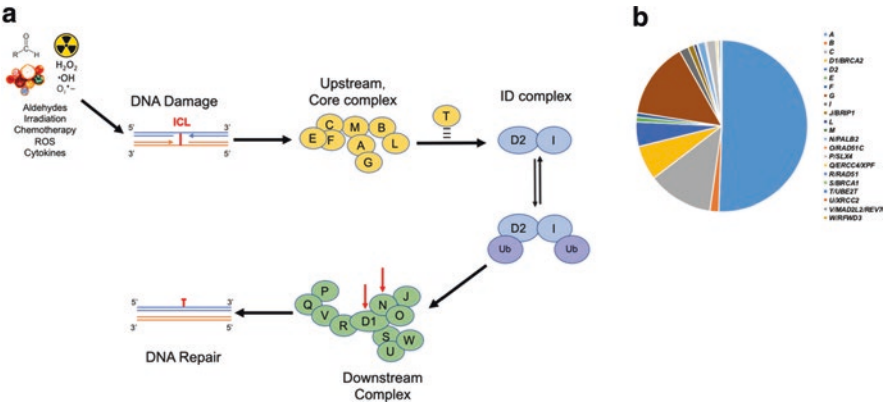

Fig. 10.2 The Fanconi anemia DNA damage response pathway. (**a**) Following DNA damage, the proteins represented by A, B, C, E, F, G, L, and M form the core complex, which is required for ubiquitination of the I and D2 proteins, which are in turn required for the downstream complex of D2-ubi, I-ubi, and D1/BRCA2, J/BACH1/BRIP1, N/PALB2, O/RAD51C, P/SLX4, Q/ERCC4/XPF, R/RAD51, S/BRCA1, T/UBE2T, U/XRCC2, V/REV7, and W/RFWD3 to form foci for DNA repair. Figure courtesy of Moises Fiesco-Roa, MD. (**b**) Relative frequencies of FA-associated genes in patients with FA reported in the literature

FANCR/RAD51, FANCS/BRCA1, FANCU/XRCC2, FANCV/REV7, and FANCW/RFWD3 to preserve genome integrity. Germline mutations in the FA genes result in markedly reduced or absent protein function and deficient DNA repair. The inability to repair DNA interstrand cross-links can lead to more DNA damage and chromosomal breakage, which are early factors in carcinogenesis. Over the last decade, accumulation of reactive oxygen species and endogenous reactive aldehyde metabolites, acetaldehyde and formaldehyde, which result from impaired detoxification by aldehyde dehydrogenase (ALDH2) and alcohol dehydrogenase (ADH5) enzymes, have been proposed as a source of intrinsic DNA damage and shown to result in accelerated bone marrow failure, leukemia, and early onset of FA in mice and human [25–28]. Alteration of cytokine homeostasis may also have roles in the pathogenesis of FA [29–31].

10.2.4 Cancer in FA

Defective DNA repair confers a propensity for certain cancers in patients with FA. In addition to BMF, patients with FA are at increased risk of MDS and AML [32, 33]. A competing risk analysis of a cohort of 163 patients with FA in the National Cancer Institute's (NCI's) cohort found the cumulative incidence of AML to reach a plateau of below 5% by 30 years of age [1]. Solid tumors, particularly SCC of the head and neck, skin, gastrointestinal tract, and genital tract, occur at higher than expected rates in FA. In that study, the cumulative incidence of solid

tumors reached 20% by age 65 years. A non-competing risk analysis found the cumulative incidence of MDS to be 50% (95% confidence interval 35–65%) by 50 years of age [1]. FA patients, particularly those who have received androgen treatment for BMF, are also at increased risk of liver tumors (e.g., hepatocellular carcinoma, liver adenomas). Patients with FA caused by biallelic mutations in *FANCD1/BRCA2* have an extremely high risk of early-onset AML, brain tumors (e.g., medulloblastoma), neuroblastoma, and Wilms tumor (a unique combination) [34, 35].

Head and neck SCC is the most common solid tumor seen in patients with FA; the risk is greater than 500-fold compared with the general population [1]. HNSCC is seen mostly in 20- to 30-year-olds, but it may be seen earlier during childhood, especially after hematopoietic cell transplant (HCT) [36].

Malignancy may be the presenting sign of FA in the absence of physical anomalies or prior family history [37, 38]. FA should be considered in a person with SCC of the head, neck, or anogenital region who is relatively young for that diagnosis, who has a limited number of other risk factors (e.g., smoking), or who has significant bone marrow suppression or other chemotherapy-related toxicities, such as mucositis. Studies of the role of human papillomavirus (HPV) have been inconsistent, but in general may be relevant for anogenital cancers but not for HNSCC [39–41].

10.2.5 Management of FA

The clinical management of patients with FA should be carefully tailored to patient-specific needs [42] (Table 10.2) and requires long-term, comprehensive care by a multidisciplinary team. Avoidance of known environmental cancer risk factors, such as tobacco and alcohol, should be emphasized. It is important to limit exposure to ionizing radiation, including occupational and medical exposures, because FA is a DNA repair disorder. MRI should be considered instead of CT scans, and consultation with a radiologist is advised for planning optimal imaging modalities.

BMF in FA is often a clinical problem very early in life; the estimated risk of BMF in FA is 50% by the age of 30 [1]. A baseline bone marrow biopsy and aspirate should be obtained at the time of diagnosis, and patients should be monitored with frequent blood counts and annual bone marrow aspirates and biopsies with cytogenetic analyses. HCT is the only current therapeutic option for cure for BMF in FA. Non-radiation-based regimens using cyclophosphamide and fludarabine for conditioning have improved the transplant outcomes. In general, HCT outcomes for FA are superior for patients transplanted prior to the development of leukemia or MDS and with a related bone marrow donor [43–49].

BMF due to FA in patients who are not candidates for HCT may be managed with anabolic steroid therapy such as oxymetholone or transfusions [50]. These patients need close monitoring of blood counts, liver function, lipids, and annual liver ultrasounds to evaluate for masses. Long-term transfusions can cause iron

Table 10.2 Clinical monitoring strategies in the IBMFS. Further recommendations for clinical care can be found at www.fanconi.org and www.teamtelomere.org. Cancer surveillance were published in 2016 by the American Association for Cancer Research's Childhood Cancer Predisposition Workshop [301, 302]

Problem	Suggested monitoring
Bone marrow failure	• Management depends on the severity. If CBCs are normal, consider an annual CBC to identify trends and early manifestations • Baseline bone marrow aspiration and biopsy with careful morphologic examination and cytogenetic studies. Consider yearly bone marrow evaluation • CBCs and bone marrow evaluation should be obtained more frequently if cytopenias, dysplastic cells, and/or cytogenetic clones and/or somatic mutations are present
Bone marrow failure—patients on androgens	• Special monitoring is required for patients on androgens for BMF • Check liver function tests prior to starting and then every 3 months • Perform liver ultrasound examination prior to initiation and semiannually for adenomas, carcinomas, or fibrosis • Check cholesterol and triglycerides prior to starting and every 6 months • Carefully follow growth and obtain baseline bone age in pediatric patients. Consider endocrinology evaluation
Cancer	• Most solid tumors develop after the first decade of life (except for FA with *FANCD1/BRCA2*) • Patients should be taught how to perform a monthly self-examination for oral, head, and neck cancer • Annual cancer screening by a dentist and a head and neck otolaryngologist. Follow oral leukoplakia carefully and biopsy any changes or suspicious sites or failure to resolve within a month • Annual gynecologic evaluation for females, including Pap smear and HPV testing • Annual dermatologic evaluation

overload, allosensitization, and allergic reactions that may complicate HCT. Patients requiring chronic packed RBC transfusions should have appropriate iron chelation.

HCT can cure the FA-associated BMF, but these patients face transplant-related problems and increased risk for malignancies compared with non-transplanted FA patients [1, 51, 52]. Successful HCT regimens extended the median survival from 20s to approximately 39 years of age, bringing a higher risk of solid tumors into adulthood. The cumulative incidence of a solid tumor in non-transplanted patients was 40% by age 40, while it was 50% by age 30 in those who had an HCT [1]. Patients with FA have a low tolerance to chemotherapy and radiation due to their intrinsic DNA repair defects, and thus wide surgical resection is recommended for solid tumors in FA [53].

Careful surveillance for solid tumors is key since early detection can reduce cancer-associated morbidity and mortality. Patients with FA should have an annual examination by an oral surgeon and/or a head and neck cancer specialist for leukoplakia or other signs of oral cavity or oropharynx SCC. They should also be taught to do a monthly self-exam of the oral cavity and lymph nodes of the head and neck. Annual gynecological examinations with visual inspection of the external genitalia

should be started at age 13, and comprehensive gynecological examinations including Pap smears are recommended beginning at age 18. Prophylactic vaccination against HPV should be offered to all FA males and females after the age of 9 in accordance with current standard guidelines [54]. Women with FA should undergo screening for breast cancer with ultrasound and MRI. There is a book with guidelines for management of FA available online (www.fanconi.org).

There are current studies for early detection, chemoprevention, and anticancer drug treatment of cancers in FA [55–57]. Gene therapy trials are ongoing with a focus on hematological abnormalities [58, 59]. Additionally, new less toxic HCT regimens and new hematopoietic stimulating agents are actively being studied. All these studies have limitations and need improvements but may become viable options in the future for the patients with FA.

10.3 Dyskeratosis Congenita (DC)

10.3.1 Clinical Features of DC

DC and associated telomere biology disorders (TBDs) include a spectrum of illnesses characterized by very short telomeres. The classical phenotype consists of the mucocutaneous triad of dysplastic finger and toenails, oral leukoplakia, and lacy, reticular skin pigmentation (Fig. 10.3, Table 10.1). However, patients may lack these mucocutaneous features, or develop them over time, which complicate the clinical diagnosis of DC/TBDs [60]. Recent advances in understanding the role of telomeres in disease have led to the recognition of a spectrum of DC-related clinical features affecting multiple organ systems. These manifestations include, but are not limited to, severe cytopenias, pulmonary and liver disease, esophageal stricture, avascular necrosis of hips and shoulders, as well as vascular anomalies such as pulmonary arteriovenous malformations and gastrointestinal telangiectasias. The most common causes of mortality in DC/TBD-affected individuals are bone marrow failure, pulmonary fibrosis, and malignancy [1, 61, 62]. Possible TBD manifestations

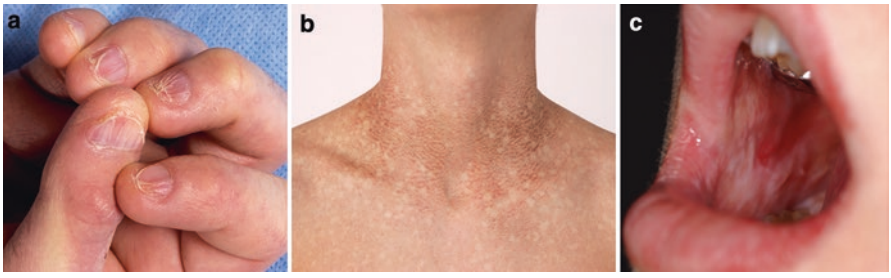

Fig. 10.3 The dyskeratosis congenita diagnostic triad. (**a**) Dysplastic fingernails; (**b**) abnormal skin pigmentation; (**c**) oral leukoplakia

range from complex multisystem disorders with onset in childhood such as classic dyskeratosis congenita (DC), Hoyeraal-Hreidarsson (HH) syndrome, Revesz syndrome, and Coats plus to patients presenting later in life with one or two DC-related features.

Patients with HH typically present in infancy with numerous complications including cerebellar hypoplasia, microcephaly, developmental delay, immunodeficiency, intrauterine growth retardation (IUGR), as well as severe bone marrow failure. The DC-associated mucocutaneous triad might not be present in very young children, but often develops in HH patients over time [63, 64]. Other HH-associated clinical features may include non-specific enteropathy and intracranial calcifications [65].

Revesz syndrome (RS) is a disorder with bilateral exudative retinopathy; IUGR; intracranial calcifications; developmental delay; fine, sparse hair; nail dystrophy; and other features which overlap with DC [66]. Coats plus is characterized by bilateral exudative retinopathy, retinal telangiectasias, IUGR, intracranial calcifications, osteopenia with tendency to fracture with poor bone healing, and gastrointestinal vascular ectasias [67–70]. Some patients may also have dystrophic nails, sparse or graying hair, and anemia. Due to the vascular ectasias, Coats plus-affected individuals are at a high risk of life-threatening gastrointestinal bleeding [70, 71].

The majority of Coats plus is caused by AR pathogenic variants in components of the CST telomere capping complex including *CTC1* [67, 69]. Identification of mutations in the Coats plus gene, *CTC1*, in DC patients revealed a common molecular etiology with telomere biology disorders [72–74].

Due to variable penetrance of expressivity of TBD-associated germline mutations (see below), patients might manifest in adulthood with one or two organ systems affected. Patients with apparently acquired aplastic anemia (AA) may actually have BMF due to a germline DC-related TBD even in the absence of DC-associated features. The acquired form of AA is usually immune-mediated and a consequence of environmental exposures, infections, or idiosyncratic reactions to medications [75]. In contrast to acquired aplastic anemia, BMF in the TBDs does not respond to immunosuppressive therapy (IST) [76].

Pulmonary complications were recently shown to occur frequently patients with DC and related TBDs, with pulmonary fibrosis being the most common manifestation [77, 78]. Idiopathic pulmonary fibrosis (IPF) is a multi-factorial disease that leads to progressive lung fibrosis and scarring. Pathogenic variants in several DC-associated genes, predominantly *TERT* and *TERC*, have been implicated in up to 20–25% of familial PF cases, but also up to 10% sporadic PF cases are associated with pathogenic variants in telomerase biology genes (*TERT*, *TERC*, *RTEL1*, *PARN*). Pulmonary function in TBDs can also be affected as part of a hepatopulmonary syndrome and might be the first presentation of portal hypertension [79]. Pulmonary arteriovenous malformation (PAVM) is increasingly recognized as part of the TBD-related phenotypic spectrum [80].

Liver disease in TBDs is complex and includes non-alcoholic, non-infectious liver cirrhosis, nodular regenerative hyperplasia, non-cirrhotic portal hypertension, and hepatopulmonary syndrome. Liver involvement may appear as the initial or sole manifestation of an underlying TBD and may also be present in patients with apparently isolated PF. Limited studies suggest that TBD-related liver disease with or without PF is predominantly associated with heterozygous germline mutations in *TERT* or, less often, in *TERC* [81].

Life-threatening gastrointestinal (GI) bleeding, mostly due to telangiectatic lesions, has recently been identified as a significant cause of morbidity in DC-associated TBDs [82]. Additional vascular abnormalities include retinal vascular disease and PAVMs.

10.3.2 Diagnosing DC and Related TBDs

The diagnosis of DC and related TBDs can be complicated due to the variable, complex, and time-dependent nature of medical problems in this spectrum of illnesses. The mucocutaneous triad is often subtle, but also progressive with age [60, 64]. Classic DC should be considered in individuals with (1) all three mucocutaneous triad features (nail dysplasia, lacy skin pigmentation, and oral leukoplakia); (2) any one feature of the triad in combination with BMF and two other physical findings consistent with DC; (3) BMF, MDS, or pulmonary fibrosis (PF) associated with a previously described pathogenic germline variant in a TBD-associated gene; or (4) two or more features seen in DC associated with telomere length below the first percentile for age [62, 83].

The unifying feature of DC-associated TBDs is the presence of very short telomeres, the result of germline mutations in key telomere biology genes (see below). Telomeres less than the first percentile for age are diagnostic of DC. Flow cytometry with fluorescent in situ hybridization (flow FISH) in leukocyte subsets is the only clinically validated test to date proven to be reliable in DC/TBD diagnostics [84–88]. Lymphocyte telomeres measured by flow FISH less than the first percentile for age are more than 95% sensitive and highly specific for differentiating patients with DC from their unaffected relatives or patients with other inherited bone marrow failure syndromes [86]. Terminal restriction fragment measurement by Southern blot, quantitative PCR, and single telomere length assays are useful in the research setting, but not yet validated for clinical diagnostics [84, 87, 89]. Telomere testing may be validated by genetic testing for germline mutations in currently known genes associated with DC/TBD (see below). However, genetic testing may be inconclusive because 20–30% of patients with classic DC do not have an identifiable genetic cause of their disease [1, 83].

10.3.3 Genetics and Pathophysiology of DC

The very short telomeres defining DC and related disorders are caused by germline mutations in genes regulating telomere maintenance (Fig. 10.4). To date, pathogenic germline variants in 15 genes (*DKC1*, *TERC*, *TERT*, *NOP10*, *NHP2*, *ACD*, *TINF2*, *POT1*, *CTC1*, *STN1*, *WRAP53*, *RTEL1*, *PARN*, *NAF1*, *ZCCHC8*) encoding for telomere biology proteins have been described to underlie DC/TBD phenotypes. DC can be inherited in XLR, AD, or AR patterns, or it can arise due to a de novo

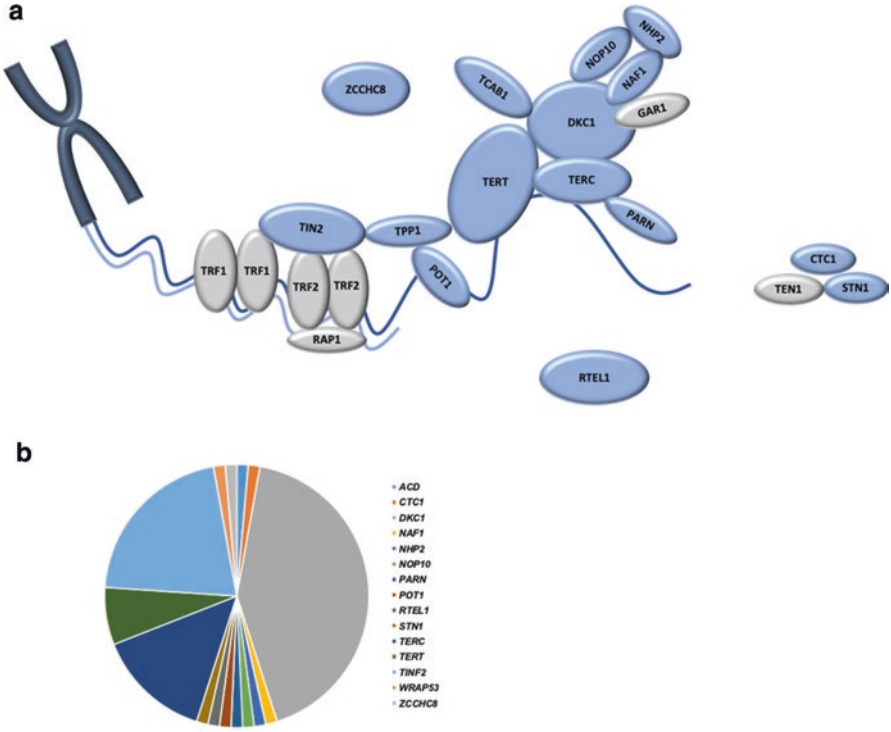

Fig. 10.4 Schematic of the telomere and the proteins affected in dyskeratosis congenita and the related telomere biology disorders. (**a**) Blue-colored shapes indicate proteins with known telomere biology disorder-associated mutations. *DKC1* dyskerin (encoding gene *DKC1*); *TERC* hTR, human telomerase RNA component (*TERC*); *TERT* human telomerase reverse transcriptase (*TERT*); *NOP10* nuclear protein family A, member 3 (*NOP10*); *NHP2* NOLA2 nucleolar protein family A, member 2 (*NHP2*); *NAF1* nuclear assembly factor 1 ribonucleoprotein (*NAF1*); *GAR1* nucleolar protein family A, member 1 (*GAR1*); *PARN* poly (A)-specific ribonuclease (*PARN*); *TCAB1* telomere Cajal body-associated protein 1 (*WRAP53*); *TPP1* telomere protection protein 1 (*ACD*); *STN1* CST complex subunit (*STN1*); *CTC1* conserved telomere maintenance component 1 (*CTC1*); *RTEL1* regulator of telomere elongation helicase 1 (*RTEL1*); *TIN2* TERF1 (TRF1)-interacting nuclear factor 2 (*TINF2*); *TRF1* telomeric repeat binding factor 1 (*TERF1*); *TRF2* telomeric repeat binding factor 2 (*TERF2*); *RAP1* TERF2 interacting protein (*RAP1*); *ZCCHC8 (ZCCHC8)* zinc finger CCHC-type containing 8. (**b**) Relative frequencies of DC/TBD-associated genes in patients with DC reported in the literature

germline mutation. DC-associated pathogenic variants show incomplete penetrance [90], variable expressivity, and genetic anticipation in successive generations [91–94].

Germline mutations in *DKC1* were first identified as the cause of XLR DC in 1998 [95]. The connection between DC and telomere length was made when the gene product, dyskerin, was shown to affect telomerase RNA. Primary fibroblasts and lymphoblasts from DC patients bearing *DKC1* mutations exhibited low levels of telomerase RNA, reduced telomerase activity, and short telomeres compared with normal controls [96].

Telomerase (*TERT*) is a reverse transcriptase that utilizes an RNA template, *TERC*. AD germline mutations in *TERC* and *TERT* can cause DC [97, 98]. The *TERT* nonsynonymous coding mutations usually lead to telomerase haploinsufficiency but can also affect enzyme processivity. Biallelic mutations in *TERT* are often associated with more severe disease [99]. *TERC* mutations usually affect the template region of *TERC*, but promoter mutations have been reported [100]. AD pathogenic variants in *TERC* frequently associate with adult onset of DC-associated manifestations, but childhood-onset, severe disease has also been identified [101]. AR DC can be the result of biallelic mutations in NOP10 or NHP2 (encoded by genes of the same names), all of which affect telomerase biogenesis [102, 103]. Recently, AD *NAF1* frameshift mutations, causing low telomerase RNA levels, were reported in pulmonary fibrosis-emphysema patients [104]. Heterozygous *PARN* mutations were first reported in familial pulmonary fibrosis, but later biallelic pathogenic variants in *PARN* were identified in patients with HH [63, 105, 106]. *PARN* mutations are assumed to destabilize TERC levels, resulting in reduced telomerase activity [107, 108]. DC can also be caused by disruption in telomerase trafficking due to AR inheritance of mutations in TCAB1 (encoded by *WRAP53*) [109]. Mutations in TPP1 encoded by *ACD*, affecting the TPP1 TEL patch, have been found to cause AD DC and AR HH [110, 111].

AD and often de novo mutations in *TINF2*, a key component of the shelterin telomere protein protection complex, also cause DC [112]. These mutations appear to disrupt the interaction between the *TINF2* protein and heterochromatin protein 1-gamma, which is required for sister telomere cohesion [113]. These variants often cause severe telomere shortening and are associated with HH and RS [114–116]. In rare cases however, *TINF2* variants may cause adult-onset pulmonary fibrosis [117–119]. Recently, biallelic *POT1* mutations were described in siblings with Coats plus [120].

Pathogenic changes in genes encoding the components of the telomere capping CST complex, *CTC1* and *STN1*, lead to impairment in duplex telomere replication and C-strand fill in [62]. *CTC1* and *STN1* alterations primarily cause Coats plus disease, which was added to the TBD spectrum after the discovery that *CTC1* mutations in AR Coats plus resulted in short telomeres and also in DC phenotypes [67, 72–74, 121, 122]. Homozygous or compound heterozygous *RTEL1* mutations are associated with very short telomeres and result in HH [123–126], while heterozygous *RTEL1* mutations were identified in pulmonary fibrosis patients [105]. RTEL1, a DNA helicase with telomeric functions, regulates telomere length, may interact

with PCNA (proliferating cell nuclear antigen), and also plays roles in DNA repair [127, 128].

Recently, a heterozygous pathogenic variant of *ZCCHC8*, encoding the zinc finger CCHC-type domain containing eight protein, was identified in a family with idiopathic pulmonary fibrosis [129]. ZCCHC8 seems to play a role in TERT maturation and subsequent telomerase function [129].

10.3.4 Cancer in DC

Patients with DC are at high risk of developing cancer [1, 130]. Analysis of the NCI's DC cohort (n = 197 patients with DC) found an approximately fourfold higher incidence of cancer in DC when compared with the general population. In patients who have undergone HCT, this risk increased to 30-fold higher [1]. In the NCI analysis, patients with DC had an increased risk of many of the same cancers as patients with FA, namely, SCC of the head and neck, and anogenital region, MDS, and AML. For DC patients, the observed/expected (O/E) ratio was 74 for any HNSCC, with an even higher ratio of 216 for tongue HNSCC. MDS, AML, and non-Hodgkin lymphoma appeared at 578-, 24-, and 11-fold greater incidences, respectively, than in the general population [1]. Due to small numbers, this study did not find a clear association between genotype and cancer. Only one study to date has evaluated the risk of cancer in patients with DC caused by mutations in specific genes [101]. Six of 30 patients with *TERC* mutations (20%) and 3 out of 17 patients with AD *TERT* (17.6%) mutations reported having had cancer compared with only 1 out of 56 (1.8%) patients with *TINF2* mutations. However, this study reported crude rates only, without age adjustment, which is important since cancer rates increase with increasing age. While both studies of cancer in DC are limited by relatively small sample sizes and the possibility of referral bias, they illustrate important connections between DC, telomere biology, and cancer.

10.3.5 Management of DC

The clinical management of DC and its related telomere biology disorders must be specifically tailored to each patient's individual medical problems (Table 10.2). The first diagnosis and management guidelines for DC and related TBDs were published in 2015 (available online at https://teamtelomere.org/resources/#research).

Clinically significant cytopenias can develop at any age in patients with DC. As in FA, BMF in patients with DC does not respond to immunosuppressive medications [131], leaving allogeneic HCT the only current opportunity to cure the bone

marrow defect. When possible, a matched, related donor HCT is the treatment of choice [132, 133], but related donors must be proven not to be affected by DC/TBD by genetic and/or telomere length testing [134]. If a matched, related donor is not available, HCT from an unrelated donor can be considered. With the increased use of reduced intensity conditioning regimen, HCT outcomes have improved in recent years [132, 135]. However, the 10-year post-transplant survival is still only between 20 and 30% [132, 135]. The to date largest, retrospective analysis of HCT data of DC patients ($n = 94$) showed better outcomes in patients with no pre-existing organ damage and in patients of younger age (3-year overall survival 72% in patients <20 years of age vs 43% in patients ≥20 years of age) [133]. Notably in this study, most patients showed irreversible lung damage post-transplant [133].

The comorbidities in DC/TBD including PF, liver disease, vascular abnormalities, and risk of secondary malignancies make the post-HCT clinical management challenging [1, 42, 77]. There is an increased risk for DC patients to develop avascular necrosis of hips and shoulders and fractures, which could be enhanced by corticosteroid use. If possible, medications known to be associated with lung or liver toxicity should be avoided. Further studies are underway to optimize HCT strategies for this unique patient group and reduce therapy-related toxicity.

Androgens may be used in patients with DC-related BMF who are not candidates for HCT. Approximately half of patients with DC appear to respond to androgens and no longer require red blood cell or platelet transfusion support [136]. Previously the oral androgens oxymetholone and halotestin have successfully been used; currently the synthetic androgen derivative danazol is preferred because it has fewer virilizing side effects [137–139]. Patients with DC may be more sensitive to androgen-related side effects, such as abnormal liver enzymes, abnormal lipid and cholesterol levels, and risk of liver adenomas [137]. Hematopoietic growth factors may be useful in BMF; however, splenic peliosis and rupture were reported in two individuals with DC who received the combination of androgens and G-CSF [140]. Hematologic surveillance in patients with DC should include CBCs (frequency based on severity of BMF), annual bone marrow aspirate and biopsy with cytogenetic analysis, and early referral for HCT.

The importance of leukoplakia as a precancerous lesion is shown by the excess in tongue HNSCC in DC patients (see above). This highlights the importance of regular surveillance and early diagnostic tests. Patients with DC should be taught to perform a monthly systematic exam for head and neck cancers. An annual ENT examination is also recommended. Baseline pulmonary function tests, with follow-up as clinically indicated, are also recommended [77]. Known environmental cancer risk factors, such as smoking and alcohol, should be avoided. In particular, smoking by patients with DC or their relatives is strongly discouraged since this is also a known IPF risk factor.

10.4 Diamond Blackfan Anemia (DBA)

10.4.1 Clinical Features of DBA

DBA is characterized by anemia, usually with normal WBC and platelet counts (Table 10.1). The isolated normochromic macrocytic anemia with reticulocytopenia of DBA is present in approximately 90% of patients within the first year of life with a median onset of 3 months of age [50]. An estimated 25% of patients with DBA have at least one congenital anomaly, although they are not as severe as in FA. Short stature is the most frequently reported observation in DBA, but the extent to which this is due to the underlying genetic defect or a side effect of corticosteroid treatment is not clear. Patients with DBA may have thumb abnormalities, such as triphalangeal, bifid, or subluxed thumbs, or subtle flattening of the thenar eminence. The radius is normal in DBA. In contrast to the other IBMFS, cleft lip and/or palate has been reported in 4% of patients with DBA, most often associated with mutations in *RPL11* [141]. Genitourinary and heart defects have been reported in up to 15% of patients [142, 143]. Occasionally, webbed neck, Klippel-Feil anomaly, and Sprengel deformity have also been seen in DBA. A recent report from the Italian DBA Registry describes more than 200 patients and outlines their phenotypic features [143]. The phenotypic spectrum of DBA is very broad, even within families. Some individuals with germline mutations may be silent carriers or have only mild anemia, whereas others are very severely affected.

10.4.2 Diagnosis of DBA

The diagnosis of DBA is based on the presence of persistent severe anemia with reticulocytopenia in the absence of other bone marrow abnormalities. The bone marrow of patients with DBA shows erythroblastopenia with normal myeloid and megakaryocytic lineages. Overall, the bone marrow cellularity is usually normal or slightly reduced. The differential diagnosis of severe anemia of childhood also includes transient erythroblastopenia of childhood (TEC). TEC usually develops in children older than 2 years of age, and most patients recover spontaneously after a few months [144].

Elevated erythrocyte adenosine deaminase (eADA) in pre-transfusion samples was first noted in DBA in 1983 [145]. A study comparing eADA levels in patients with DBA with other IBMFS patients showed that eADA had a sensitivity of 84%, specificity of 95%, and positive and negative predictive values of 91% [146]. That study also noted that 16% of classical clinical DBA patients had a normal eADA. Thus, if the eADA is elevated, it supports the diagnosis of DBA, but, if it is normal, it does not exclude the diagnosis.

10.4.3 Genetics and Pathophysiology of DBA

DBA is predominately an autosomal dominant disease caused by pathogenic germline variants in genes encoding ribosomal proteins [147]. Haploinsufficiency involving 19 ribosomal genes encoding key components of the small 40S (*RPS7*, *RPS10*, *RPS15A*, RPS17, RPS19, *RPS24*, *RPS26*, *RPS27*, *RPS28*, *RPS29*) or large 60S (*RPL5*, *RPL11*, *RPL15*, *RPL18*, *RPL26*, *RPL27*, *RPL31*, *RPL35*, *RPL35A*) ribosomal subunits has been well established in DBA etiology (Fig. 10.5) [147, 148]. Additionally, two X-linked genes, *TSR2* and *GATA1*, have also been implicated in the pathogenesis of red cell aplasia in infancy. *TSR2* encodes for a ribosome chaperone, and *GATA1* encodes for a hematopoietic transcription factor targeted by altered ribosome levels [149–151]. Traditionally 50–60% of DBA cases have been found to harbor a pathogenic variant in one of the ribosomal genes; however, this number has increased to 70–80% in more recent studies [143, 147, 152]. *RPS19* was the first gene discovered to cause AD DBA and the first link between DBA and ribosomal biogenesis [153]. *RPS19*, *RPL5*, *RPS26*, and *RPL11* are the most commonly mutated genes in DBA with *RPS19* mutations making up about 25% of all DBA cases [143, 147, 150]. In addition, de novo mutations occur; due to variable disease penetrance, family members may also be clinically "silent" carriers of mutations.

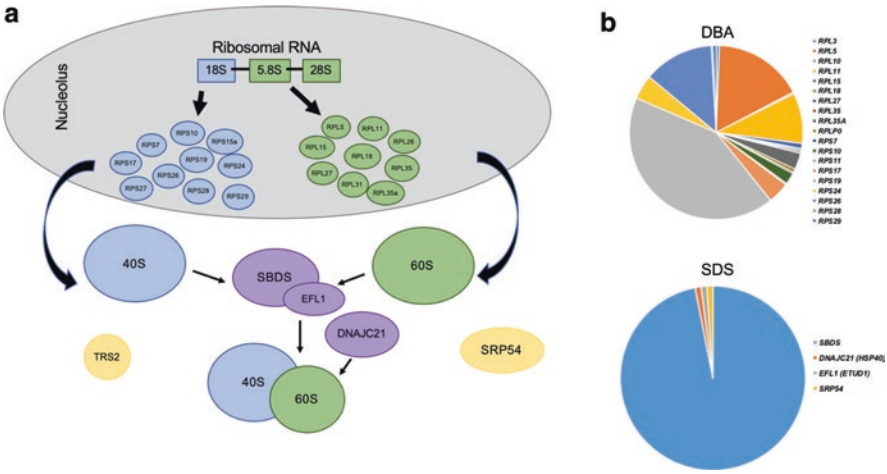

Fig. 10.5 The ribosomal biogenesis pathway. (**a**) Pathways involved in ribosomal synthesis link the biology of Diamond Blackfan anemia (DBA), Shwachman Diamond syndrome (SDS), and dyskeratosis congenita (DC). The genes encoding the ribosomal protein components are mutated in DBA and affect 40S and 60S ribosome biogenesis. *DKC1* encodes the dyskerin protein, implicated in ribosomal RNA pseudouridylation (Ψ) and the cause of X-linked recessive DC. The SBDS protein affected in SDS appears to be involved in the joining of the 40S and 60S ribosomal subunits to form the mature 80S ribosome. (**b**) Relative frequencies of DBA and SDS-associated genes based on patients reported in the literature

Most of the DBA-associated germline mutations result in abnormal assembly of ribosomal proteins. Ribosome assembly is a highly regulated process, and the specific defect in erythropoiesis is thought to be mediated by p53. Zebrafish models using antisense morpholinos targeting *rps19* and other ribosomal genes manifested impaired erythropoiesis and developmental malformations [154, 155]. These phenotypes in both mice and zebrafish could be at least partially rescued by knocking out p53. One model, as suggested by these animal studies, is that disruption of ribosomal biogenesis activates stress signaling pathways such as p53 to result in apoptosis or cell cycle arrest of erythroid progenitors or in the developing embryo (reviewed in [156, 157]). More recently, a zebrafish model that expressed a novel *TP53* germline variant from a patient with DBA-like symptoms was found to have increased p53 activity, impaired erythrocyte differentiation, and decreased erythroid production [158]. The leading hypothesis is that haploinsufficiency for ribosomal proteins results in increased p53 levels and, via a MDM2-mediated mechanism, leads to apoptosis of erythroid precursors and cell cycle arrest [159]. However, the pathogenesis of non-anemia-related phenotypes that are common in DBA is still unclear (e.g., short stature, thumb abnormalities).

Initially, germline *GATA1* mutations were identified in X-linked dyserythropoietic anemia and thrombocytopenia, a distinct disorder from DBA [160]. However, identification of X-linked mutations in *GATA1* families with apparent DBA further expanded understanding of its role [150]. *GATA1* encodes a key component of the GATA family of transcription factors that is important in erythroid development. Notably, the red cell ADA levels were normal in the DBA patients with *GATA1* mutations [150]. The finding of germline *GATA1* mutations in multiple families with DBA expands the biological basis of DBA etiology and connects it to the related, yet distinct, X-linked dyserythropoietic anemia and thrombocytopenia [147, 150, 161–164].

10.4.4 Cancer in DBA

Early case reports and a case series suggested that DBA patients were predisposed to develop AML and MDS [165]. In addition to AML and MDS, other reports suggested increased risk of osteosarcoma [165, 166]. Another review identified reports of AML, MDS, and solid tumors, including osteosarcoma, in literature cases [50]. The DBA Registry of North America (DBAR) conducted the first quantitative risk assessment of cancer in DBA in 2012, with an update in 2018. This prospective study of patients with DBA was established in 1991; the most recent update included data on 702 patients [167, 168]. Overall, patients in the DBAR have a 4.8-fold increased risk of cancer compared with the general population [1, 168]. The highest risk was for MDS with an observed-to-expected (O/E) ratio of 352. The O/E ratios for AML, osteosarcoma, colon carcinoma, and vaginal squamous cell carcinoma were 29, 42, 45, and 172, respectively. In patients in the DBAR, the cumulative incidence of any type of cancer was 13.7% by 45 years of age. The large Italian

DBAR reported that the most frequent cancer was osteosarcoma in patients with mutated *RPS19* [143].

The molecular pathogenesis of this increased cancer risk is not yet established. However, it is possible that the aberrant ribosomal biogenesis that results from the germline ribosomal protein gene mutations activates cellular stress signaling pathways, such as p53 [169–171]. This change in p53 balance could disrupt cellular homeostasis and result in increased cancer risk.

10.4.5 Management of DBA

Transfusions of RBC are the primary treatment modality for severe anemia until the diagnosis of DBA is firmly established [50, 148, 172, 173]. At that point, oral corticosteroids may be considered to treat the anemia. They have been used successfully for decades in DBA and can minimize or even eliminate the need for RBC transfusions. Most patients respond to an initial dose of prednisone of 2 mg/kg/day, which can be tapered to a lower dose and an every-other-day schedule with fewer side effects. However, some patients may require higher doses or lose their initial response. The side effects of corticosteroids need to be carefully balanced with the erythroid response. Details with regard to management and tapering of steroids are provided in the consensus guidelines [172] and a recent review [174]. Steroids are generally avoided during the first year of life because of impaired growth side effects [148]. Approximately 40% of patients who initially respond to steroids become steroid-dependent, and those who fail to respond to corticosteroids require chronic red blood cell transfusions or HCT [147, 148]. Patients with DBA on a chronic transfusion regimen typically require a red blood cell transfusion every 3–5 weeks, and iron overload due to multiple RBC transfusions is a major problem. Iron chelation with subcutaneous desferrioxamine or oral deferasirox should be initiated early. It should be noted that approximately 20% of patients with DBA may develop a treatment-free remission from either steroids or transfusions [168]. The Italians noted that this occurred only in those with mutations in *RPS* genes, suggesting that *RPL* genes were associated with a more severe disease [143].

HCT is the only current curative modality for the anemia of DBA. Making the decision to undergo HCT is difficult since only one hematopoietic lineage is affected. Indications for HCT include steroid refractory anemia, chronic red blood cell transfusion dependence, or aplastic anemia [148, 175]. Details on HCT modalities and outcomes in DBA have been previously reported [172, 176]. In general, patients with matched sibling donors are preferred, but siblings need to be screened for DBA to ensure they are not asymptomatic carriers [148, 175]. The impact of nonmyeloablative compared with ablative HCT conditioning regimens is unclear due to a limited number of available studies; however, an expert panel recommended a standard myeloablative conditioning regimen with busulfan or treosulfan for patients with DBA that meet criteria for HCT [175].

Patients with DBA should be informed of the increased cancer risk associated with the disorder. Blood counts should be performed at least every 6 months or as clinically indicated. Bone marrow aspirate with biopsy and cytogenetic studies should be performed if the hemoglobin, white blood cells, or platelets fall rapidly. Bone marrow karyotype and FISH to look for acquired abnormalities in chromosomes 5, 7, and 8 should be utilized since these chromosomal abnormalities are associated with MDS/AML [172]. There are no proven surveillance modalities specific for solid tumors associated with DBA. Patients should be advised to seek treatment early for any medical concerns.

10.5 Shwachman Diamond Syndrome (SDS)

10.5.1 Clinical Features of SDS

SDS is an autosomal recessive disorder, initially described by gastroenterologists and hematologists based upon exocrine pancreatic insufficiency and BMF (often primarily neutropenia) [177]. The exocrine pancreatic insufficiency usually presents in infancy with failure to thrive and steatorrhea. This may lead to poor growth and malnutrition if not recognized. Short stature and certain skeletal abnormalities, such as metaphyseal dysostosis, osteopenia, or delayed bone age, may be present. The hematologic abnormalities in SDS can vary, but typically involve persistent or intermittent neutropenia; thrombocytopenia and/or anemia may also be present.

10.5.2 Diagnosis of SDS

SDS is diagnosed based on the combination of exocrine pancreatic insufficiency and single- or multi-lineage cytopenia (Table 10.1). SDS is the second most common cause of inherited pancreatic insufficiency after cystic fibrosis [178]. Serum trypsinogen levels are usually low in patients with SDS but may improve with age. Serum isoamylase levels are consistently low, whereas, normally, levels rise until age 3 years [179]. Exocrine pancreatic insufficiency can be determined by the presence of elevated fecal fat, but this is not specific for pancreatic dysfunction and, since malabsorption can improve over time, normal values do not preclude the diagnosis.

The hematologic manifestations of SDS include low levels of at least one myeloid lineage. It is important to evaluate blood counts over several months to establish trends for each individual patient because of variability in cytopenias. The bone marrow may show varying degrees of hypocellularity, maturation arrest, and/or evidence of myelodysplasia. The clinical severity of SDS varies, but additional features such as short stature, skeletal abnormalities (particularly metaphyseal dysostosis),

dental abnormalities, and/or hepatomegaly, with or without decreased of serum transaminases, can further support the diagnosis [178].

10.5.3 Genetics and Pathophysiology of SDS

SDS is inherited in an AR manner in at least 90% of patients. However, there are more males than females reported [180]. It is caused by germline mutations in the Shwachman-Bodian-Diamond syndrome (*SBDS*) gene located at the centromere on chromosome 7q11 (Fig. 10.5). The majority of patients have one allele with mutations in exon 2 of the *SBDS* gene (usually c.258 + 2T > C) [181]. These mutations include exon deletions, gene conversions, intronic changes, and gene rearrangements. The clinical laboratory performing the mutation detection must be equipped to evaluate for these types of mutations.

The SBDS protein is a key component of ribosome biogenesis. It associates with the 60S ribosomal precursor but not with the mature 80S ribosome. SBDS is found in both the nucleus and the cytoplasm of the cell and appears to move in and out of the nucleolus based on the cell cycle. SBDS has also been implicated in additional molecular pathways including mitotic spindle stabilization [182], cellular stress response [183], actin dynamics [184], and signaling downstream of RANK for osteoclast differentiation [185].

Recently three new genes have been described for SDS and an SDS-like syndrome. Biallelic mutations in *DNAJC21* were identified in families with clinical SDS, but lacking *SBDS* mutations [186, 187]. DNAJC21 is a heat shock protein and interacts with the 45S ribosomal RNA and other 60S ribosomal maturation factors. In a similar cohort of patients with SDS, but lacking *SBDS* mutations, biallelic mutations in *EFL1* identified that as another SDS causing gene [188–190]. EFL1 cooperates with SBDS to catalyze the release of eIF6 and activate translation in the ribosome. The third new gene identified is *SRP54* and was described in patients with SDS-like features. This is the first autosomal dominant form of the disorder. SRP54 interacts with the signal recognition particle receptor to target the ribosome and endoplasmic reticulum [191].

10.5.4 Cancer in SDS

The connection between AR mutations in the SBDS gene and cancer is not yet understood. SBDS is key to numerous cellular processes, and thus its perturbation may be related to the carcinogenic process. MDS/AML is the primary cancer of concern in patients with SDS. The NCI's cohort study had 35 SDS patients of whom

6 developed MDS and 1 developed AML; this is significantly increased over the expected rates when matched for age, sex, and birth cohort (O/E 200 and 7700) [1]. The most frequent cytogenetic clones in SDS are i(7)(q10) and del(20)(q), but they are not necessarily harbingers of AML. In fact, i(7)(q10) specifically duplicates the mild mutation 258 + 2T > C, and cells with this may have a selective growth advantage and not develop leukemia [192]. Cells with del(20)q also have a selective growth advantage due to loss of EIF6 [193]. Mutations in *TP53* have also been associated with SDS-related MDS [194]. Myers et al. report a multicenter retrospective cohort of 37 patients with SDS who developed MDS or AML. They documented poor outcomes in these patients from both severe treatment-related mortality and disease resistance [195]. There is not good evidence for solid tumors in patients with SDS, although there are single case reports of dermatofibrosarcoma protuberans [196], early-onset breast cancer [197], pancreatic adenocarcinoma [198] and ovarian cancer [1], and two lymphomas [199, 200].

10.5.5 Management of SDS

The management of SDS should be based on each patient's specific needs. A gastroenterologist should follow patients with SDS due to the presence of fat malabsorption [201, 202]. Administration of oral pancreatic enzymes and fat-soluble vitamins, A, D, E, and K, are the mainstay of treatment for malabsorption. A pediatric endocrinologist should follow bone development and growth. Metaphyseal dysostosis may become painful with age, and joint replacement is sometimes indicated [203].

Frequent monitoring of blood counts is important due to the high rates of neutropenia in patients with SDS. Neutropenic patients with fever need to be promptly evaluated and managed with appropriate cultures and antibiotics. Treatment of SDS-associated neutropenia with granulocyte colony-stimulating factor (G-CSF) is used in patients with active bacterial infections. G-CSF is also sometimes initiated based on neutrophil counts alone [204]. The potential association of leukemia with the dose of G-CSF may not be causal; high doses of G-CSF may have been given to patients who did not have an improvement of their neutrophil count because of an intrinsic problem with the neutrophils [190].

HCT for SDS is generally reserved for patients with severe, prolonged cytopenias, MDS, or AML. The optimal timing of HCT and the ideal transplant regimen are not known. HCT in patients with SDS and leukemia may be complicated by prolonged cytopenias and end-organ dysfunction [205, 206]. As with any inherited disorder, potential sibling donors should be evaluated for the pathogenic mutations identified in the proband.

10.6 Severe Congenital Neutropenia (SCN)

10.6.1 Clinical Features of SCN

Patients with SCN develop severe bacterial infections, such as pneumonia or abscesses, early in infancy (Table 10.1). These patients may present in the neonatal period with omphalitis. Skin infections, including deep abscesses, as well as diarrhea, pneumonia, and deep abscesses in the liver, lungs, and subcutaneous tissues are common in the first year of life. These patients do not have characteristic birth defects that could aid in diagnosis. In SCN, the neutrophil count is very low, often less than 0.5×10^9/L (<1.5×10^9/L is required for the definition), on multiple occasions as well as in the setting of an infection. The hemoglobin and platelet counts are usually normal [207, 208].

10.6.2 Diagnosis of SCN

The diagnosis of SCN is based on the combination of frequent, severe bacterial infections early in infancy and profound neutropenia. The bone marrow of these patients shows a maturation arrest at the promyelocyte/myelocyte state. The bone marrow cellularity is usually normal or may be slightly reduced. The other cell lineages are normal. It is important to differentiate SCN from cyclic neutropenia [207, 208]. In cyclic neutropenia, the neutrophil counts vary over an approximately 21-day cycle. SCN can be differentiated from cyclic neutropenia by obtaining white blood counts (WBCs) and absolute neutrophil counts (ANC) twice a week for 6 weeks; in SCN, the counts are consistently very low.

10.6.3 Genetics and Pathophysiology of SCN

The initial report by Kostman in 1956 described severe neutropenia of infancy occurring in an AR inheritance pattern [209]. Subsequent descriptions of clinical cases and the discovery of causative genes have broadened the etiologic spectrum in SCN. Heterozygous mutations in neutrophil elastase, encoded by *ELA2* (*ELANE*), cause approximately one-half of SCN [210]. These are inherited in an AD manner or occur as de novo mutations in the proband. Neutrophil elastase is a critical serine protease component of the neutrophil's primary azurophilic granules. Another form of AD SCN is caused by mutations in growth factor-independent 1 transcriptional repressor (*GFI1*), a transcriptional target of ELA2 [211].

Loss-of-function AR mutations in *HAX1*, a mitochondrial protein with homology to the BCL2 family of proteins, cause the form of SCN originally described by Kostman [212] and account for about 15% of patients in the SCN International

Registry [213, 214]. HAX1 is important in maintenance of the inner mitochondrial membrane potential but has many other cellular functions, and its role in SCN is not completely understood. It has been postulated that activation of the apoptotic caspase cascade plays a role in SCN.

An AR form of SCN is caused by mutations in glucose-6-phosphatase catalytic subunit 3 (*G6PC3*), a component of the glucose-6-phosphate pathway. This gene was discovered as a cause of SCN associated with intermittent thrombocytopenia, cardiac defects, and a prominent superficial venous pattern in a large consanguineous family. Subsequent reports described patients with similar features and homozygous mutations in *G6PC3* [215]. Additionally, X-linked mutations in *WAS* can cause isolated SCN, as well as Wiskott-Aldrich syndrome [214, 216]. *WAS* mutations result in defective actin polymerization that affects numerous cell types. One mutated gene mentioned above for SDS is *SRP54* and is the second most common in the French CN Registry. Bone marrow showed dysgranulopoiesis, with large cytoplasmic vacuoles in promyelocytes. Inheritance was AD or de novo mutations. Patients did not respond to G-CSF, none had acquired mutations in, and despite high doses none transformed to leukemia [217]. These genetic subgroups of SCN are reviewed in detail by others; the list of causal genes and syndromes now has two dozen candidates although 20–30% of cases with SCN do not have mutations in the known genes [207, 213, 214, 218–220].

10.6.4 Cancer in SCN

MDS/AML is the primary cancer of concern in patients with SCN. This was first reported in individuals with SCN prior to the availability of G-CSF treatment, suggesting an underlying genetic etiology [221–224]. Prospective data on 374 patients with SCN on long-term G-CSF found that the overall risk of MDS/AML was 15–25% at 15 years on treatment. Higher doses of G-CSF (above the median of 8μg/kg/day) were used to achieve (or usually failed to do so) adequate neutrophil counts, associated with an increased risk of death from sepsis or MDS/AML [204, 225]. This was hypothesized to be due to a defective (and leukemia-prone) stem cell, not to the G-CSF per se. Notably, acquired mutations of the gene *CSF3R*, encoding the G-CSF receptor, have been associated with leukemic progression in SCN [226].

10.6.5 Management of SCN

The prognosis for patients with SCN was very poor prior to the advent of G-CSF therapy [227]. Most patients died due to severe bacterial infections and sepsis. G-CSF is now used to increase the neutrophil counts to $>1.5 \times 10^9$/L [228]. The response to G-CSF is varied. Some patients require high doses and thus may be at

increased risk of SCN-associated MDS or leukemia (perhaps not driven by the G-CSF but reflecting nonresponsive stem cells as described above).

Blood counts should be monitored very closely in patients with SCN. A bone marrow aspirate with biopsy and cytogenetic analyses should be performed if there are significant changes in the blood counts or at least at the time of diagnosis to confirm the clinical presentation and distinguish various phenotypes (e.g., maturation arrest in classical CN, myelokathexis in WHIM syndrome, hemophagocytosis in autoimmune neutropenia, abnormal granules in Chediak-Higashi disease) [219]. Patients who fail to respond to G-CSF and are at risk of infection and/or leukemia should be considered candidates for hematopoietic stem cell transplantation. This consideration was put into action by the French Severe Congenital Neutropenia Registry, in which the indication for HCT was extended beyond MDS-AL to include requirement for high doses of G-CSF (>15μg/kg/day), and the authors suggested that this policy lowered the rate of leukemia [229].

10.7 Congenital Amegakaryocytic Thrombocytopenia (CAMT)

10.7.1 Clinical Features of CAMT

Patients with congenital amegakaryocytic thrombocytopenia (CAMT) usually present in infancy with petechiae or serious hemorrhages but do not have characteristic birth defects [230, 231]. Since the severity of the thrombocytopenia can vary, this disease is likely under-recognized. Some patients with CAMT may evolve to BMF or even MDS without prior recognition of the underlying thrombocytopenia.

10.7.2 Diagnosis of CAMT

CAMT should be considered in the differential diagnosis of patients with unexplained thrombocytopenia, especially during infancy (Table 10.1) [230, 231]. The platelets in CAMT are typically normal in size and morphology. The platelet count may be in the 20,000/μL range, but higher platelet counts do not rule out the diagnosis. The bone marrow of patients with CAMT may have decreased or absent megakaryocytes. The overall bone marrow cellularity is normal but can decrease with time.

10.7.3 Genetics and Pathophysiology of CAMT

The majority of patients with CAMT have AR biallelic mutations in the *MPL* gene. *MPL* encodes the thrombopoietin (TPO) receptor, an essential regulator of megakaryopoiesis and platelet production [232, 233]. Two forms of CAMT have been described based on the type of mutation in *MPL*. Group 1 patients have frameshift or nonsense mutations in *MPL* that completely disrupt TPO receptor signaling and typically a more severe disease phenotype with persistent, severe thrombocytopenia and early development of pancytopenia. Group 2 patients have missense *MPL* mutations, which may lead to reduced but not absent receptor function. These patients often have a milder disease phenotype with rising platelet counts in the first year of life and delayed BMF [230, 231, 234].

10.7.4 Cancer in CAMT

Patients with CAMT may be at increased risk of hematologic malignancies due to dysregulation of TPO production. There is emerging evidence that *MPL* is a proto-oncogene. Myeloproliferative disease (MPD) and MPD-like leukemia were found in mice with MPL mutations causing constitutively activated *MPL* protein [235]. Ectopic expression of MPL in mouse models result in BMF with skewed differentiation and induction of MPD [235].

A retrospective analysis of 20 patients with CAMT found that 14 patients developed pancytopenia. One of these had refractory anemia with excess blasts (10% blasts in the bone marrow) and a cytogenetic clone (trisomy 21) [234]. The Israeli IBMFS registry reported data on eight CAMT patients from six families. There was one patient with MDS and one with acute lymphoblastic leukemia (ALL) [236]. Patients with CAMT may develop or present with aplastic anemia; the underlying CAMT may not have been recognized [219]. There is one report of osteosarcoma following bone marrow transplantation; the preparative irradiation was suggested as the cause [237].

10.7.5 Management of CAMT

Platelet transfusions are usually reserved for patients with CAMT who also have bleeding symptoms. Prophylactic platelet transfusions are not recommended unless there is a high risk of bleeding (e.g., prior to surgery). Antifibrinolytic agents may be used for mucous membrane bleeding. Occasionally, desmopressin acetate (DDAVP) is used in patients with thrombocytopenia with careful monitoring for side effects such as the syndrome of inappropriate antidiuretic hormone secretion.

HCT is the only curative modality for CAMT. Sibling donors should be tested for biallelic *MPL* mutations. Considerations for timing of transplant include minimizing the risk of allosensitization from donor blood products or infectious complications secondary to neutropenia. The reader is referred to comprehensive reviews for additional information [230, 231].

10.8 Thrombocytopenia Absent Radius Syndrome (TAR)

10.8.1 Clinical Features and Diagnosis of TAR

TAR syndrome is typically diagnosed in infancy due to thrombocytopenia with bilateral absence of radii with the presence of thumbs (albeit abnormal) (Table 10.1) [238, 239]. Patients may have additional bony abnormalities of the ulna or humerus. Occasionally, hip and/or patellar dislocation and other non-specific bony abnormalities are present. The thumbs are always present in TAR, in contrast to FA where the radial ray abnormality results in missing thumbs if radii are absent [239, 240]. Approximately 10% of individuals with TAR are reported to have congenital heart disease, and structural renal anomalies occur in about 7% [238, 241–243]. Additionally, bloody diarrhea or enteritis associated with cow's milk intolerance has been reported in about 20% of cases [240, 244]. The majority of patients with TAR present with thrombocytopenia and platelet counts less than 50,000/μL. The thrombocytopenia in TAR syndrome is generally transient; it is usually present during infancy and childhood and significantly improves with time, but usually does not reach normal levels [245].

10.8.2 Genetics and Pathophysiology of TAR

TAR is primarily due to biallelic AR inheritance of a deletion at chromosome 1q21.1 from one healthy parent and a single nucleotide polymorphism (SNP) in the 5' untranslated region (rs139428292) or intron 1 (rs201779890) of the RNA binding motif protein 8A gene (*RBM8A*) on the non-deleted 1q21.1 allele from the other parent [246, 247]. Inheritance of two hypomorphic variants in *RBM8A* has also been reported to cause TAR [247]. A report of siblings with TAR-like dysmorphology identified the rare variant rs61746197 in an NFkB-p65(RelA) transcription factor binding motif, but not in *RBM8A*, as disease associated, suggesting a wider range of associated phenotypes in this genomic region [248].

10.8.3 Cancer in TAR

The risk of cancer in patients with TAR syndrome is not known. Transient leukemoid reactions have been reported in some individuals [249]. There are four case reports of leukemia developing in patients with TAR [250, 251]. One of those patients was included in the NCI IBMFS cohort study that reported this patient as the only case with TAR and leukemia out of a total of five TAR syndrome patients [252].

10.8.3.1 Management

The management of thrombocytopenia in patients with TAR syndrome is generally supportive with platelet transfusions if needed for bleeding or surgery. Blood counts are followed as needed, on a clinical basis. Because the thrombocytopenia is usually transient, HCT is not usually indicated but has been reported [240, 245, 253, 254]. Orthopedic surgery and physical therapy can be used to maximize the function of the upper limbs after the platelet count improves.

10.9 Inherited Disorders Associated with Myelodysplastic Syndrome and/or Acute Myeloid Leukemia

Over the past two decades, an increasing number of disorders with inherited predisposition to myeloid malignancy have been identified (Table 10.3). Most of these disorders predispose patients to early-onset myelodysplastic syndrome (MDS) with conversion to acute myeloid leukemia (AML), but some present with de novo AML. Additionally, while most have been described in children, adolescents, and young adults, there has been a growing understanding among adult clinicians that germline inherited predispositions can present in adulthood. The understanding of these syndromes has identified the major causes of pediatric MDS. Here we will highlight a few of these rare disorders (see also Chap. 11).

10.10 GATA2 Deficiency

GATA2 is zinc finger transcription factor that binds to the consensus sequence W/GATA/R (W = A or T and R = A or G) in the promoter regions of downstream target genes critical for hematopoiesis and vascular development. GATA2 is essential for normal hematopoiesis and lymphatic vascular development. Detailed reviews on this topic can be found elsewhere [255, 256].

Patients with GATA2 deficiency have predisposition to myeloid malignancy along with a constellation of other symptoms. Several groups described the disorder each giving it a different name (Emberger syndrome, MonoMAC syndrome, familial MDS/AML, DCML deficiency) before it was understood that the underlying cause was germline mutations in *GATA2* and is now termed GATA2 deficiency (Table 10.3) [257–262]. GATA2 deficiency is an autosomal dominant disorder, and de novo mutations are not infrequent. Presentation of the disorder can vary widely, and this is possibly due to variable expressivity and penetrance of *GATA2* mutations and specific yet-to-be-identified genotype-phenotype correlations.

GATA2 deficiency is characterized by severe monocytopenia, NK cell and B cell lymphopenia, and low numbers of dendritic cells. These cytopenias often evolve to MDS (84%) and can transform to AML (14%). Patients may also present outright with aplastic anemia or CMML. This multisystem disease includes severe infections with nontuberculous mycobacteria, pulmonary alveolar proteinosis, disseminated fungal and viral infections, primary lymphedema of the lower extremities, and sensorineural deafness [261, 263]. Among pediatric MDS, 7% of patients have GATA2 deficiency, and among adolescents with MDS with monosomy 7, 37% have the disease [264, 265]. Patients should be monitored closely for the development of MDS. Pre-emptive HCT is often used for patients with cytopenias and/or other organ dysfunction.

10.11 Familial Platelet Disorder with Associated Myeloid Malignancy: Germline *RUNX1* Mutation

Patients with familial platelet disorder with associated myeloid malignancy (FPDMM) have platelet dysfunction, mild to moderate thrombocytopenia, and increased risk of MDS and AML (Table 10.3) [266]. Platelet dysfunction is thought to be due to a dense granule storage pool deficiency, and combined with thrombocytopenia, this puts patients at bleeding risk. Interestingly, some patients have been noted to have severe eczema of unclear etiology. This rare disorder is caused by autosomal dominant (AD) inheritance of germline mutations in *RUNX1* (previously called *AML1* or *CBFA2*) [267, 268]. RUNX1 is part of a family of transcription factors that share homology for a region called the "runt homology domain" (RHD) [269]. *RUNX1* encodes one subunit of a heterodimeric transcript factor that controls hematopoietic genes. Thus, it is a key regulator of hematopoiesis and myeloid differentiation. Aberrant regulation of *RUNX1* is often seen in sporadic MDS and AML [266, 270].

The AD mutations in *RUNX1* are usually in the N-terminus of the protein in exons 3–5 and disrupt DNA binding of RUNX1 to target genes, but allow its dimerization with core binding factor (CBF) [266–268, 270]. Less commonly, germline *RUNX1* mutations occur in the C-terminus and result in loss of the trans-activation region but maintain DNA binding and dimerization. The penetrance of AML in

Table 10.3 Features of autosomal dominant inherited syndromes primarily associated with MDS and AML

Disorder	Clinical features	Biological pathway	Known genes
Familial AML with mutated *CEBPA*	Non-specific	HSC differentiation	*CEBPA*
Familial platelet disorder	Bruising or bleeding due to platelet dysfunction	Transcription factor involved in HSC regulation and differentiation	*RUNX1*
GATA2 deficiency (Emberger syndrome, MonoMAC)	Monocytopenia; severe infections with nontuberculous mycobacteria; reduced NK, B, and dendritic cells; pulmonary alveolar proteinosis; lymphedema	Transcription factor involved in development and proliferation of hematopoietic, vascular, and endocrine cell lineages and otic development	*GATA2*
MIRAGE Ataxia-pancytopenia	Infections, growth restriction, adrenal hypoplasia, genital malformations, enteropathy, cerebellar ataxia	Cell proliferation, apoptosis	*SAMD9*, *SAMD9L*
MECOM-associated syndrome	Amegakaryocytic thrombocytopenia, radioulnar synostosis	Transcriptional regulation of hematopoiesis	*MECOM* (*EV1* and *MDS1* locus)

Abbreviations: *MDS* myelodysplastic syndrome; *AML* acute myeloid leukemia; *HSC* hematopoietic stem cell

individuals with AD *RUNX1* mutations is highly variable but estimated between 20 and 60% [271]. The reported age of onset ranges from 6 to 75 years in several pedigrees [271, 272]. Patients with FPD have also been reported to develop T-cell acute lymphoblastic leukemia. Patients with FPD should have blood counts monitored based on the degree of platelet function and thrombocytopenia [273]. The risk of other cancers and medical problems is not known in these individuals.

10.12 Familial AML: Germline *CEBPA* Mutation

CEBPA encodes the CCAAT/enhancer binding protein alpha (C/EBPα) and is a basic region-leucine zipper transcription factor important for the differentiation of granulocytes. It is often mutated in somatic leukemia cells [274]. C/EBPα inhibits cellular proliferation and has tumor suppressor activity. The most common mutations result in a truncation of the protein and a dominant negative effect. This results in the formation of progenitor cells with deregulated proliferation and higher rates of transformation [275]. The germline mutations are generally located in the N-terminal region with the transactivating domains, while the somatic events tend to be toward the C-terminus contained the leucine zipper [276].

AML with somatic biallelic *CEBPA* mutations has a relatively favorable prognosis, and thus, somatic mutation testing has been integrated into clinical care [277–279]. Individuals with AML and biallelic mutations in their leukemic cells should undergo germline mutation testing of *CEBPA*. This can be done ideally on skin fibroblasts or a blood sample obtained when in complete remission.

Germline *CEBPA* mutations are inherited in an AD manner and have nearly complete or complete penetrance for development of AML (Table 10.3) [278, 280–283]. In these families, the age of onset of AML can be highly variable and is reported from 4 to 50 years of age. The prognosis is more favorable for individuals with germline *CEBPA* mutations and a normal somatic AML karyotype (overall survival 50–65%) than in individuals without a germline *CEBPA* mutation and a normal somatic AML karyotype (overall survival 25–40%) [282, 284]. Some studies suggest that this improved prognosis primarily occurs in individuals with biallelic *CEBPA* mutations [275, 285]. These patients are at risk for recurrence of disease with a new leukemic clone even years after initial remission and thus need to be monitored closed after a first leukemia [276, 286]. The optimal AML surveillance regimen for patients with germline *CEBPA* mutations is not known. The risk of subsequent cancers or presence of other medical problems in these individuals is not known.

10.13 MIRAGE and Ataxia Pancytopenia: Germline *SAMD9* and *SAMD9L* Mutations

SAMD9 and *SAMD9L* are genes in tandem on chromosome 7. Autosomal dominant gain-of-function mutations in these genes lead to overlapping, but distinct, phenotypes in patients. These mutations lead to an increase in SAMD9 and SAMD9L anti-proliferative effect. Mutations in both lead to cytopenias and a predisposition to myeloid malignancy often with monosomy 7 [287]. Approximately 30% of pediatric MDS patients with monosomy 7 will be found to have a germline mutation in *SAMD9* or *SAMD9L* [288]. As *SAMD9* and *SAMD9L* are both on chromosome 7, reversion of the mutated allele through copy neutral loss of heterozygosity, uniparental isodisomy, or somatic loss-of-function mutation in cis has been observed in blood lineages leading to recovery from cytopenias [287, 289]. This is important to consider when performing germline testing as the germline mutation may appear somatic. Patients with *SAMD9* mutations present in infancy with MIRAGE syndrome which includes myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy [290]. Most are de novo mutations in the affected individual. In contrast, patients with mutations in *SAMD9L* can present anytime from infancy through adulthood and have ataxia, cerebellar atrophy, alveolar proteinosis, and infections. Both patients with *SAMD9*- and *SAMD9L*-associated syndromes are at risk for MDS and AML. Currently, there is discussion on the best treatment for these patients and the role of HCT.

10.14 Other Inherited Predispositions to MDS and AML

Several other genes have been associated with predisposition to myeloid malignancy. Biallelic mutations in *ERCCL62* lead to a relatively newly described bone marrow failure disorder with predisposition to MDS and AML [291, 292]. Some, but not all, of the reported patients have developmental delay and microcephaly. Autosomal dominant mutations in *SRP72* have been associated with bone marrow failure and development of MDS, as well as auditory abnormalities [293]. Mutations in three genes cause a combination of thrombocytopenia and predisposition to myeloid malignancy—*DDX41*, *ETV6*, and *ANKRD26*. *DDX41* has recently been noted to be common in adults with previously unrecognized inherited disease [294]. Germline *ETV6* mutations have also been associated with acute lymphoblastic leukemia. Patients with *ANKRD26* mutations are noted to have hypogranular platelets in addition to the thrombocytopenia. Heterozygous autosomal dominant mutations in *MECOM* (*EVI1* and *MDS1* locus) lead to radioulnar synostosis with amegakaryocytic thrombocytopenia and bone marrow failure [295, 296]. While MDS and AML have yet to be reported in these inherited patients, this locus is known to be involved in sporadic AML [297].

10.15 Genetic Testing, Education, and Counseling

The advent of rapid, high-throughput whole-exome and whole-genome sequencing has led to the identification of the genetic etiology in a growing majority of patients with inherited disorders [298–300]. For example, in DBA, the gene was known in only about half of patients 10 years ago and now is up to nearly 70–80% in recent studies [143, 147, 152]. This explosion in new genomic techniques over the last two decades has led to new understanding of the importance of germline predisposition to bone marrow failure, MDS, and myeloid malignancies and to the discovery of new syndromes. Importantly, these discoveries also illustrate the clinical overlap of some classic inherited bone marrow failure syndromes with immunodeficiencies (e.g., DC and HH).

This highlights the importance of considering germline predisposition when evaluating a patient with a new cancer diagnosis, planning treatment, choosing an HCT donor, and family counseling.

Genetic education and counseling are essential for all families undergoing genetic testing. Individuals or the parents of young children should have some understanding of the genetics and biology behind the disorder for which they are being evaluated. Genetic testing results have implications for the individual, first-degree relatives, and the extended family because carriers who have not yet manifested disease may be identified. Individuals undergoing testing need to understand that future medical complications cannot be predicted but many complications can

be monitored. Reproductive decision-making may or may not change with the knowledge of a germline genetic disorder in an individual's family.

Detailed phenotypic and molecular studies of patients and their family members are critical to improve our understanding of the clinical consequences and underlying disease etiology. This work has the potential to improve genotype/phenotype/outcome associations, which in turn will lead to better ability to prognosticate and treat.

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Chapter 11
Inherited Risk for Childhood Leukemia

Jennie Vagher, Luke Maese, Amanda Gammon, Wendy Kohlmann, and Joshua D. Schiffman

Abstract Childhood leukemia is the most common pediatric malignancy, diagnosed in nearly a third of pediatric cancer patients. Awareness and identification of the hereditary component of childhood leukemia continues to increase. Childhood leukemia, especially acute lymphoblastic leukemia (ALL), has been attributed to a dysregulated immune system with different patterns of infectious exposure at a young age. Candidate gene studies have revealed inconsistent associations with specific SNPs related to folate metabolism, xenobiotic metabolism, DNA repair, immunity, and B-cell development. More recently, genome-wide association studies (GWAS) have demonstrated stronger associations with SNPs in B-cell development genes and increased risk for childhood ALL. Identical twins have been described with childhood leukemia due to presumed placental transfer, but non-twin siblings with childhood leukemia also have been very rarely reported suggesting a genetic link to disease susceptibility. In this chapter, we review the known hereditary cancer syndromes associated with leukemia which can be divided into six categories: (1) DNA repair syndromes, (2) RASopathies, (3) bone marrow failure syndromes, (4) immunodeficiency syndromes, (5) germline predisposition to leukemia, and (6) congenital syndromes.

Keywords Childhood leukemia · Acute lymphoblastic leukemia (ALL) · Acute myeloblastic leukemia (AML) · Inherited cancer syndromes · Genetic · Infection · Genome-wide association study (GWAS) · Siblings · Familial · Genetic instability · DNA repair · Cell cycle/differentiation · Bone marrow failure · Telomere biology disorder · Immunodeficiency · Predisposition to familial leukemia

J. Vagher · A. Gammon · W. Kohlmann
Family Cancer Assessment Clinic, High Risk Cancer Research Clinics, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA

L. Maese · J. D. Schiffman (✉)
Family Cancer Assessment Clinic, High Risk Cancer Research Clinics, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA

Department of Pediatrics, University of Utah, Salt Lake City, UT, USA
e-mail: Joshua.Schiffman@hci.utah.edu

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11.1 Introduction

Childhood leukemia is the most common pediatric cancer and accounts for over a third of all new cancer diagnoses in children and adolescents [1–3]. Leukemia can be classified by the hematopoietic progenitor cell, with nearly 80% of all pediatric diagnoses defined as acute lymphoblastic leukemia (ALL). ALL further can be classified by immune cell phenotype as B-cell ALL (the most common) and T-cell ALL (less common and typically more aggressive). The other leukemia phenotypes typically seen in pediatric patients include acute myeloid leukemia (AML) and the rarer infant ALL diagnosed in children less than 1 year of age. Chronic leukemias, also quite rare in the pediatric population, occur as well. As combination chemotherapy regimens have evolved, outcomes for childhood leukemia have improved tremendously over the past five decades with current overall survival for pediatric B-cell ALL now approaching 90% [4]. In contrast, the overall survival for pediatric AML is somewhat lower, approaching 60%, with little improvement realized in the past several decades [5–7]. The etiology of childhood leukemia has remained an area of active investigation for many decades. While more evidence has begun to accumulate for the genetic contribution to leukemia risk as a small fraction of childhood leukemia cases, up to 4% are attributed to pathogenic variants and/or known hereditary cancer syndromes [8]. Although low penetrant risk alleles have been described that may contribute to the risk of childhood leukemia, true pathogenicity is yet to be elucidated. This chapter will explore the known genetic risk factors for childhood leukemia, including the associated hereditary cancer syndromes.

11.2 Epidemiology and Leukemogenesis

Over 3250 new cases of childhood acute leukemia are diagnosed each year in the United States, with 1 in 2000 children at risk for ALL in the normal, healthy population [9, 10]. The majority of leukemia in children and adolescents less than 20 years old will be the ALL subtype, with the most common age of diagnosis between 2 and 6 years [1, 3, 9]. As age increases, incidence of AML also increases, and AML is more common than ALL in the elderly. Both ALL and AML are slightly more common in boys (the male/female incidence is 1.2:1 and 1.1:1, respectively) [9]. Leukemia is most common in Caucasian populations [9]. Many case-control studies have demonstrated that increased birth weight is a risk factor for ALL [11, 12].

Many theories exist as to the causes of childhood leukemia, although a single unifying theory has yet to explain all cases [13]. Nevertheless, many investigators have demonstrated both epidemiological and biological evidence that the risk of developing ALL may correlate with a dysregulated immune response to infection [13]. The Kinlen Population Mixing Hypothesis states that the rare demographic mixing of a novel virus between susceptible and infected individuals in the perinatal period may trigger leukemogenesis [14, 15]. The Delayed Infection Hypothesis

(Greaves Hypothesis) argues that delayed exposure to a common infection following underexposure during infancy leads to leukemogenesis due to an evolutionary mismatch between immune system programming and modern (hygienic) lifestyle [16]. Although based on slightly different key notions, both hypotheses involve the child's abnormal response to an infectious agent. Greaves and colleagues have demonstrated the presence of pre-leukemic clones in dried newborn blood spots at birth [17]. Furthermore, Greaves and colleagues have convincingly demonstrated that the high concordance rate of ALL with shared identical translocation breakpoints in monozygotic twins is due to blood cell chimerism via vascular anastomoses within a single or monochorionic placenta [18]. This has led to a model of childhood leukemogenesis that follows a combination of chance, exposure, and inherited genetic variation, leading to in utero initiation, followed by postnatal promotion and finally full-blown ALL [1]. Within the current understanding of the leukemogenesis genomic landscape, only a small percentage of the time is the process accelerated by identifiable pathogenic variants. It is unclear how the current COVID-19 pandemic will impact rates of childhood leukemia going forward [19].

11.3 Sibships and Maternal Contribution

Published studies report an inconsistent association between childhood leukemia and a family history of cancer. A recent international collaboration of 54 sibships with 2 or more cases of childhood ALL found a surprisingly high ALL subtype concordance within sibships, incompatible with all cases of ALL in the sibships having occurred randomly or sporadic [20]. In contrast, two death certificate studies [21–23], a US Childhood Cancer Survivor Study cohort study [24], and a large Nordic population- and registry-based study revealed only a very moderate increased risk of ALL among siblings [25]. Other reports have documented a nearly threefold increased risk in childhood ALL with a positive family history of cancer [26–28], while others only show a borderline increased risk [29, 30]. Pre-leukemic subclones have been demonstrated in the newborn blood spots of *ETV6-RUNX1 (TEL-AML1)*, high-hyperdiploid, *MLL*-rearranged, and a few other ALL subsets [18, 31–36]. Taken together with the high ALL subtype concordance rates within sibships, this most likely reflects shared genetic and/or environmental risk factors. Other less likely and more speculative scenarios include (1) the transfer of leukemic cells to the mother during pregnancy [37] and then subsequent transmission to a fetus [38, 39] and (2) failure of maternally driven subtype-specific immune-protective mechanism against recurrence of ALL.

Evidence of a maternal contribution to ALL risk includes a recent report of a mother of two siblings with concordant hyperdiploid B-cell ALL, who was found to carry a rare allele in the *PRDM9* gene responsible for meiotic recombination [40]. The same *PRDM9* allele was then found in significant excess in a validation cohort of parents ($N = 44$) and childhood B-cell ALL patients ($N = 50$), suggesting that *PRDM9* may play a role in genomic instability related to leukemogenesis [40].

Coupled with the finding of *TP53* germline pathogenic variants in patients with childhood leukemia [41, 42] (see below), it is possible that inherited susceptibility to genomic instability and deficient DNA repair lead to increased clonal heterogeneity in normal hematopoietic and pre-leukemic stem cells increasing the risk for leukemogenesis.

11.4 Candidate Gene and Genome-wide Association Studies

Investigators have endeavored for many years to try and find genetic etiologies for childhood leukemia [43]. The earliest investigations explored candidate genes associated with the biology of ALL and included five main categories of (1) folate metabolism/transport, (2) xenobiotic metabolism/transport, (3) immune function, (4) DNA repair, and (5) cell cycle [44, 45]. Hundreds of studies have been published with mixed results, but the candidate genes that seem to be most suggestive of an association of ALL risk are *MTHFR* C677T (folate metabolism) [46–54], *CYP1A1* TP235C (xenobiotic metabolism) [55, 56], *GSTM1* deletion (xenobiotic metabolism) [56–61], NAT2*5 (xenobiotic metabolism) [57, 62–65], *XRCC1* G28152A (DNA repair) [66, 67], and HLA-DRB4 (encoding HLA-DR53 immune antigen) [68–70].

More recently, investigators have used a more agnostic approach combined with single-nucleotide polymorphism (SNP) microarrays to perform genome-wide association studies (GWAS). These GWAS have consistently identified SNPs annotating the *IKZF1* (7p12.2), *CDKN2A* (9p21.3), *ARID5B* (10q21.2), and *CEBPE* (14q11.2) genes [71–77], which are associated with growth regulation, hematopoiesis, and lymphocyte development—all pathways that clearly would be involved in the functional onset of childhood ALL. These risk alleles have been validated in children with ALL of European descent and are among the strongest cancer susceptibility variants identified through any GWAS with a nearly threefold risk of disease [78]. This suggests a relatively large impact of inherited genetic factors on the pathogenesis of childhood ALL [79], and it is estimated that these SNPs account for approximately one third of ALL risk conferred by common genetic polymorphisms [79]. The first ALL GWAS in a multiethnic population (including African Americans and Hispanic Americans) compared susceptibility loci across ethnic groups and demonstrated novel susceptibility variants at the *BMI1-PIP4K2A* locus [80]. Validation studies have confirmed these results in Hispanic children with ALL and noted the association with *ARID5B*, *CEBPE*, and *BMI1-PIP4K2A* variants and hyperdiploid subtype [81, 82]. These GWAS provide clear evidence of the power of unbiased genomic strategies in ALL to identify genes of central importance in leukemogenesis. However, the loci reported in ALL GWAS thus far have accounted for only 8% of genetic variation in ALL risk [79], suggesting that additional susceptibility variants have yet to be discovered.

11.5 Inherited Syndromes

Both ALL and AML have been associated with a variety of hereditary cancer syndromes, although leukemia is rarely seen as the only presenting cancer. The categories of leukemia-associated inherited cancer syndromes can be divided into six main categories, four of which include predisposition to solid tumors as well, (1) DNA repair syndromes, (2) RASopathies, (3) bone marrow failure syndromes, (4) immunodeficiency syndromes, (5) germline predisposition to leukemia, and (6) congenital syndromes. We will discuss each category separately, although overlap exists between them.

11.6 DNA Repair Syndromes

Li-Fraumeni Syndrome (TP53). Li-Fraumeni syndrome (LFS) was first described in 1969 in four families with soft tissue sarcoma and early-onset breast cancer [83] and was later demonstrated to be due to germline *TP53* pathogenic variants [84]. LFS is a highly penetrant hereditary cancer syndrome with an estimated prevalence of 1/5000 to 1/20,000 though some recent estimates suggest that it could be as common as every 1/500 [85–87]. *TP53* codes for the p53 protein, which is mostly responsible for DNA repair and apoptosis. Families with LFS may develop any type of cancer, and it was soon recognized that leukemia, mostly ALL and to a lesser extent myeloid malignancies (AML and myelodysplastic syndrome), was part of the LFS clinical phenotype. Of the nearly 1250 germline *TP53* pathogenic variants described in the International Agency for Research on Cancer (IARC) TP53 database (www.iarc.p53.fr), only 3.37% (42 germline variants) have been associated with hematological malignancy [88]. Published cohorts also report leukemia as part of LFS at a range of 1–5% [86, 89–91]. It is unknown if germline *TP53* pathogenic variants predispose to certain subtypes of leukemia, although, recently, a strong association was shown between pediatric patients diagnosed with hypodiploid ALL and underlying germline *TP53* pathogenic variants. In fact, in a large case series of patients with hypodiploid ALL, nearly half were found to harbor germline *TP53* pathogenic variants [42]. In this case series, family history was not collected, and *TP53* variants were not confirmed on fibroblast samples. Another report describes familial leukemia with hypodiploid childhood ALL in the setting of an undiagnosed germline *TP53* pathogenic variant [41]. In 2018, the Children's Oncology Group published the germline and somatic data for ALL clinical trials, AALL0232 and P9900 [92]. Consistent with previous research, this study also identified an association between *TP53* variants and hypodiploid ALL. Additionally, inferior overall survival was reported for those with germline TP53 variants due to the increased risk to develop a second malignancy. It is now clear that a substantial portion of patients with hypodiploid ALL may have underlying germline *TP53* pathogenic variants. Another association between relapsed ALL and LFS has been identified,

suggesting that 1–2% may have an underlying diagnosis of LFS [93]. As LFS consortiums are established, the connection between germline *TP53* pathogenic variants and leukemia risk (and subtype) will become better elucidated [94]. Annual complete blood counts (CBCs) were initially suggested as part of biochemical screening for early leukemia detection in LFS [95], although this has currently fallen out of favor without evidence that early detection of leukemia in LFS changes clinical outcome [96]. Screening for leukemia with CBCs in LFS is still debated as two out of the four LFS screening guidelines do not recommend CBC [96]. It is less controversial to monitor for early signs of myelodysplasia for patients who received leukemogenic agents for the treatment of their first malignancy [96, 97].

Constitutional Mismatch Repair Deficiency Syndrome (MLH1, *MSH2*, *MSH6*, *PMS2).* Monoallelic pathogenic variants in the four mismatch DNA repair genes *MLH1*, *MSH2*, *MSH6*, and *PMS2* have been well described in association with Lynch syndrome, or hereditary non-polyposis colon cancer (HNPCC) [98]. Lynch syndrome is associated with an increased lifetime risk of early cancers including 52–82% for colorectal cancer, 25–60% for endometrial cancer, 6–13% for gastric cancer, and 4–12% for ovarian cancer [98]. Biallelic (homozygous or compound heterozygous) pathogenic variants in the mismatch repair genes were found in patients with multiple café-au-lait spots, pediatric brain tumors, and pediatric hematological malignancies including both ALL and AML [99–103]. Patients with constitutional mismatch repair deficiency (CMMRD) syndrome, also known as biallelic mismatch repair deficiency syndrome, were shown to demonstrate an ultra-hypermutated state of tumorigenesis with a rapid burst of simultaneous mutations of nearly 600 mutations per cell division [104, 105]. The population prevalence of Lynch syndrome has been estimated to be between 1 in 279–440 [106, 107]; therefore, the incidence of CMMRD would be approximately 1 in one million with gene-specific incidences reported [107, 108]. CMMRD is underdiagnosed in the pediatric population and therefore may have a higher than recognized prevalence [109]. To date, this remains an unusual syndrome in children, with 56 CMMRD patients with hematological malignancies from 48 families reported in the literature and summarized in a recent publication [110]. Hematologic malignancies have been reported in one third of CMMRD patients, with the most common subtypes being non-Hodgkin lymphoma (mostly mediastinal and T cell in origin), T-cell ALL, and AML [110, 111]. Primary, secondary, and tertiary hematologic malignancies have been reported [110]. The actual leukemia penetrance is unknown in CMMRD, although it is estimated to be quite high. Any child presenting with ALL, NHL, or AML with multiple café-au-lait spots and/or a family history of Lynch syndrome-related tumors should be considered for genetic testing. Specific criteria for CMMRD testing consideration have been created, which includes a point system in which NHL of T-cell origin diagnosed under age 18 is part of the criteria [108]. If CMMRD is identified in a pediatric patient, this will have clinical implications for both parents who are generally obligate carriers of monoallelic MMR defects and would thus have Lynch syndrome. There are currently no standard screening recommendations for leukemia in patients with CMMRD, but awareness of clinical features of leukemia should be reviewed with parents. In 2017, two consensus statements were created for

CMMRD management, and both recommended CBC every 6 months starting at 1 year of age; however, both cite the limited evidence about the effectiveness of surveillance for leukemia [109, 112].

Bloom Syndrome (BLM). The *BLM* gene encodes for a helicase instrumental in double-stranded DNA break repair [113]. Lymphocytes from patients with Bloom syndrome (BS) show characteristic cytogenetic changes, including high frequency of sister chromatid exchanges and quadriradial configurations, illustrating the high level of genetic instability caused by the syndrome [114, 115]. A *BLM* founder mutation exists in the Ashkenazi Jewish population, c.2207_2212delinsTAGATTC, with an estimated carrier frequency of 1/100 [114, 116]. The hallmarks of BS include severe growth deficiency, erythematous facial skin lesion, and a high propensity for malignancy [115, 116]. BS had previously been diagnosed by cytogenetic analysis of sister chromatid exchanges (SCE), noting that those with BS have increased SCE, up to tenfold of those without BS [117, 118]. Recently there have been reports of other syndromes exhibiting a similar rate of SCE as those with BS, so genetic testing for BS diagnosis is now standard of care, except in cases where biallelic BLM pathogenic variants are not identified [119, 120]. Given the rarity of BS (less than 300 cases reported), much of this disorder's information comes from case reports as well as the Bloom Syndrome Registry maintained by Dr. James German [121]. The risk for malignancy in patients with BS is high with approximately 33.4% developing cancer by age 25, and that number increases to 80% by age 40. The average age of cancer onset is 25 years old (range 4–44 years old) [116, 122]. A wide range of cancers have been described in Bloom syndrome patients including common solid tumors, rare solid tumors, and hematologic malignancies (both lymphoma and leukemia). Forty cases of leukemia were documented among the 277 registered Bloom syndrome patients ($N = 17$ AML, 11 ALL, 12 other/biphenotypic/unspecified); both primary and secondary leukemias have been reported [123]. Median age of diagnosis was 18 years though it ranged from ages 2 to 40, and it is worthwhile mentioning that cases of AML have been reported until age 47 [118]. Of those who were diagnosed with secondary leukemias, myelodysplasia usually preceded the diagnosis, and it is likely that the excess of AML cases reported in BS is thought to be treatment related [118, 124]. Monosomy 7 is a frequent somatic cytogenetic finding in AML that occurs in Bloom syndrome patients [125, 126]. MDS has also been reported in patients with Bloom syndrome [126, 127]. Expert recommendations based on the Bloom Syndrome Registry have recently been published which include comments on leukemia surveillance and mitigation of secondary leukemia risk for BS patients. Annual CBC and bone marrow biopsy/aspirate are not recommended. Rather counseling for symptom awareness and work-up for leukemia if symptoms appear has been the guidance. There are additional recommendations made for the treatment of other malignancies to reduce the risk to develop secondary malignancies. For example, individuals with BS are exceptionally sensitive to radiation treatment and chemotherapy in addition to radiation exposure from CT and PET scans. Thus, patients with BM should utilize MRI and ultrasound for imaging, reduce chemotherapy dosage (50% or below normal dosage), and avoid ionization radiation and alkylating agents when possible [124].

Werner Syndrome (WRN or RECQL2). Werner syndrome (WS) is an AR disorder characterized by premature aging and short stature. Growth slows in late childhood, with most individuals reaching their final height between ages 10 and 18 [116]. Premature aging features of Werner syndrome start appearing between ages 20 and 40 [116, 122]. Cancer, atherosclerosis, and diabetes are the most common causes of death, occurring at much younger ages than seen in the general population [128]. WS results from loss-of-function pathogenic variants in *WRN* which cause inactivation of a RecQ helicase [122, 129]. Consistent with the early aging phenotype of Werner syndrome, affected individuals are at increased risk for early-onset and multiple primary cancers. The general incidence of malignancy development in WS has been reported to be 14% [130]. A 2013 literature review by Lauper et al. assessed the types of neoplasms reported in 189 individuals with WS ($N = 248$ neoplasms). The average age of neoplasm onset was 43.3 years old, and 22% of the patients developed multiple neoplasms [122]. The six most reported neoplasms were thyroid (16.1%), malignant melanoma (13.3%), meningioma (10.9%), soft tissue sarcomas (10.1%), hematologic/lymphoid (9.3%), and osteosarcoma/bone (7.7%) [122]. Of note, when calculating the standard incidence ratios for these WS-related neoplasms compared to the general Japanese population (using the total estimated WS population in Japan), the leukemia risk in WS was not significantly elevated over average [122]. The most common type of hematologic malignancy in WS is AML, although T-cell ALL and erythroleukemia also have been reported [122, 128, 130]. Preleukemic marrow disorders (myelofibrosis, myelodysplasia, refractory anemia with excess blasts) and plasmacytoma have also been seen [122, 128, 129]. No specific leukemia surveillance recommendations have been offered for patients with WS.

Rothmund-Thomson (RECQL4). Rothmund-Thomson syndrome (RTS) is characterized by prenatal growth deficiency, characteristic skin lesions, prematurely gray and sparse hair, and an increased risk for juvenile cataracts and malignancy [116]. It is a rare disorder with fewer than 400 patients reported in the literature [131, 132]. It results from biallelic pathogenic variants in the gene coding for the *RECQL4* helicase causing DNA repair dysfunction. It should be noted that two other conditions are also associated with biallelic pathogenic variants in *REQL4*: RAPADILINO and Baller-Gerold syndrome [132]. Pathogenic variants in *RECQL4* can be identified in up to 60% of patients with clinical features of RTS [131, 132]. Diagnosis is generally made clinically based on physical examination with the characteristic RTS rash as a classic physical finding and/or with the identification of *REQL4* pathogenic variants through genetic testing, if available [133]. The most common cancers associated with RTS are osteosarcoma and skin cancers [134]. A study of 31 patients with clinical diagnoses of RTS identified 11 patients (35%) with osteosarcoma (median age at diagnosis was 9 years, with a range of 4–20 years) [135]. Among 61 reported patients with RTS who developed malignancy, 16 developed skin malignancies (26%, mean age 34.4) including 3 squamous cell carcinomas of the tongue [134]. Very few patients with RTS and hematological malignancy have been reported, so the incidence of leukemia in RTS is unknown. A few patients with RTS have been reported to have MDS, as well as one patient with AML, one with non-Hodgkin lymphoma, and one with neutropenia and leukopenia [134, 136].

Individuals with RTS are also at risk to develop multiple primary malignancies; for example, a patient with multiple primary malignancies was reported to have large cell anaplastic T-cell lymphoma, diffuse large B-cell lymphoma (centroblastic variant), osteosarcoma, and T-cell ALL [131, 134]. No leukemia screening recommendations have been proposed for patients with RTS, probably due to rarity of disease and unclear leukemia risk. Currently available guidelines for RTS screening only mention concern for solid tumor malignancy, such as osteosarcoma and skin cancers [137].

11.7 RASopathies

Neurofibromatosis Type 1 (NF1). The *NF1* gene codes for neurofibromin, which functions as a negative regulator of the RAS signal transduction pathway. Pathogenic variants in NF1 lead to neurofibromatosis type 1 (NF1), an inherited genetic syndrome characterized by multiple café-au-lait spots, axillary and inguinal freckling, multiple cutaneous neurofibromas, iris Lisch nodules, bony dysplasias, learning disabilities, and cancer risks that include optic pathway gliomas, astrocytomas, malignant peripheral nerve sheath tumors, and leukemias (including chronic/juvenile myelomonocytic leukemia [CMML/JMML], MDS, AML, and ALL) [138, 139]. The prevalence of *NF1* is quite high at 1:3000 individuals, and pathogenic variants can be equally inherited or de novo [140, 141]. Although leukemia is not the primary malignancy associated with NF1, the relative risk (RR) is still quite high with one longitudinal 17-year study from the United Kingdom reporting CMML RR of 221 (95% CI 71–514) and ALL RR of 5.4 (95% CI 2.8–9.4) [142]. More recently, another study identified a RR for chronic myeloid leukemia in those with NF1 to be about 6.7. The increased risk for chronic leukemia is consistent with a RR of at least above 2 cited by the Stiller (above) and Matsui papers [143, 144]. Monosomy 7-related MDS has been reported in 11% of a small cohort of NF1 patients ($N = 64$) and increased to 75% (6 of 8) among patients treated for pediatric embryonal cancer [145]. There is a 200–500-fold increased risk for JMML, and 10–14% of children with JMML may have a clinical diagnosis of NF1 [146, 147]. Those with JMML and NF1 are often diagnosed later, after 5 years of age, and have greater frequency of thrombocytosis with an elevated blast percentage in the bone marrow when compared to other JMML subtypes [148]. No formal leukemia screening recommendations exist for children with NF1, although bruising, petechiae, and fatigue should raise a high index of suspicion for leukemia in this population [149]. If a child with NF1 has juvenile xanthogranulomas, then this child should be assessed for risk to JMML [150]. Any child with JMML should be carefully examined for clinical signs of NF1 as the JMML diagnosis could be the initial manifestation of NF1 [147].

Noonan Syndrome (PTPN11, SOS1, KRAS, NRAS, RAF1, BRAF, SHOC2, or MEPK1). Noonan syndrome (NS) is an AD disorder that is part of the family of syndromes involving the RAS pathway. Due to the multiple distinct physical features seen in NS, it is typically identified in childhood through a comprehensive

physical exam followed by confirmatory genetic testing. NS is caused by pathogenic variants in *PTPN11*, *SOS1*, *KRAS*, *NRAS*, *RAF1*, *BRAF*, *SHOC2*, or *MEPK1*; of note, only 70–75% of individuals with NS have an identifiable pathogenic variant in one of these genes [151]. *PTPN11* pathogenic variants are the most common cause of NS, accounting for approximately 50% of cases [152]. In addition to specific dysmorphology, individuals with NS often have cardiac defects (pulmonary valve stenosis, hypertrophic cardiomyopathy, atrial septal defect), developmental delay, and cognitive impairment [116]. Children with NS are predisposed to a variety of hematologic abnormalities, which include JMML. Specific pathogenic variants in PTPN11 and KRAS have been shown to be associated with a myeloproliferative disorder (MPD) that resembles JMML and presents as either transient or fulminant [153–156]. Unlike children without NS, this MPD/JMML may spontaneously resolve or follow a more aggressive clinical course resembling JMML [153]. Kratz et al. published a cohort of 632 individuals with molecularly confirmed NS and found that there was approximately an eightfold increased risk for malignancy with JMML ($N = 4$), brain tumor ($N = 2$), ALL ($N = 2$), and neuroblastoma ($N = 1$) [157]. In a 2011 literature review, Kratz et al. assessed reported cancers in 1151 published cases of NS [151]. Cancers had been reported in 45 of these individuals, including ALL ($N = 8$), neuroblastoma ($N = 8$), glioma ($N = 6$), and rhabdomyosarcoma ($N = 6$) [151]. Recent consensus surveillance guidelines published have recommended that those with specific PTPN11 or KRAS pathogenic variants (known to be associated with MPD/JMML) should undergo physical examinations with spleen size assessment and CBC every 3–6 months starting at birth until age 5. The authors also comment on the lack of evidence for a survival advantage when screening as recommended, though given the possibility of an aggressive course of the MPD/JMML, this may justify the recommendation. The authors also comment on the value of consulting with JMML experts in the event that MPN/JMML is diagnosed as treatment may be the preferred course of action despite the possibility of spontaneous resolution [156].

CBL Syndrome (CBL). CBL syndrome (CBLS) is the most recently described leukemia-related RASopathy and primarily causes JMML, along with specific dysmorphic features [158, 159]. CBLS was identified in 2009, and since its discovery, the prevalence of this condition remains unknown. Those with CBLS display clinical overlap with other RASopathies (i.e., NS and NF1), including a relatively high frequency of neurologic features/vasculitis and mild NS features. The defining feature of CBLS is identification of a germline pathogenic variant in the *CBL* gene. Inheritance is AD, although multiple de novo cases have been reported [158, 159]. Approximately 10–15% of those with JMML are expected to have CBLS, and risk to develop JMML is expected to be high but not precisely defined among those with CBLS [156, 160]. In 2 separate reviews of JMML cohorts of 142 and 132 patients, *CBL* variants were identified at a frequency of 9 and 18%, respectively [161, 162]. Children with JMML and CBL variants are often found to have a germline *CBL* variant on one allele and an acquired loss of heterozygosity on the other allele in leukemic cells [163]. JMML seen in CBLS often spontaneously resolves, but it may take an aggressive clinical course [163, 164]. Becker et al. published a patient who

developed AML and was subsequently found to have CBLS; it remains unknown whether those with CBLS are predisposed to developing other myeloid malignancies outside of JMML [165]. Consensus surveillance guidelines for JMML in CBLS have been published. These propose that screening should start at birth with a physical examination, spleen size assessment, and CBC with differential, every 3–6 months until age 5 [156].

11.8 Bone Marrow Failure Syndromes (see also Chapter 10)

Fanconi Anemia (Over 16 complementation groups, including FANCA-C, FANCD1–2, FANCE-G, FANCI-J, and FANCL-P). Fanconi anemia (FA) is usually an AR disorder (with the exception of *FANCB*-related Fanconi anemia, which demonstrates X-linked inheritance). *FANCA* pathogenic variants account for the majority of FA cases (60–70%) [166, 167]. FA is a chromosomal breakage disorder caused by defects in DNA repair. The primary features of FA include bone marrow failure, distinct physical characteristics, growth failure, and increased risk for AML/MDS and solid tumor malignancies. FA is typically diagnosed in childhood through a combination of dysmorphology examination and chromosomal breakage studies, though approximately 25–40% of those with FA do not have any physical features [168]. Blood lymphocytes from those with FA show distinctive chromosomal aberrations when cultured with a DNA crosslinking agent such as diepoxybutane (DEB) or mitomycin C (MMC), so much so that this has become the gold standard of diagnostic testing for FA [166, 167, 169]. FA diagnosis can be complicated given the chance of somatic mosaicism and high genetic heterogeneity [166, 170]. Aplastic anemia in FA generally occurs in childhood between ages 5 and 15 years old, though marrow failure and other myeloid malignancies have been reported in adulthood [171, 172]. In a 2014 study, types and frequencies of cancer reported in FA patients were analyzed in published literature between 1927 and 2012. Of the 2000 cases with FA in the literature, 188 had a diagnosis of leukemia, and 84% of the reported leukemias were AML. Further analysis of this data identified the relative risk to develop AML to be 700-fold and the relative risk to develop MDS to be 6000-fold [173]. Acute lymphocytic leukemia has been reported in FA, though the majority of diagnoses are confined to the *BRCA2* (FANCD1) subtype; those with biallelic IVS7 pathogenic variants in *BRCA2* have been specifically shown to cause a substantially increased risk for AML [174]. In an analysis of four cohorts with FA, a cumulative incidence of AML was 15–20% by age 40, and incidence of MDS reached 40% by age 50 [175]. Clonal chromosomal abnormalities (1q gain, 3q gain, and 7 loss) have shown to occur more frequently in those with FA who have AML or MDS. The risk for developing any hematologic abnormality is 90% by age 40. Despite the high risks for MDS and AML, no standard therapies have been establish for those with FA [174]. There is a substantial risk for other solid tumors, such as SCC of the head and neck, and the risk for solid tumors is likely increased following bone marrow transplant, although this may represent a survival bias [166, 174, 176]. Individuals

with FA are highly susceptible to DNA-damaging agents including radiation. Treatment for FA-related malignancy is often de-intensified from standard of care due to the high rate of secondary malignancy. Those with heterozygous pathogenic variants in *PALB2* and *BRCA2* have increased risks for cancers, especially female breast cancer, and recommendations for early cancer detection are widely available through NCCN [177]. This represents another reason to identify complementation groups in those with FA. Due to the high risk of AML/MDS in FA, periodic monitoring of the peripheral blood and bone marrow is important. Annual evaluation of the bone marrow, starting as early as 2 years of age, including aspirate with or without biopsy and cytogenetics is recommended. Of note baseline marrow dysplasia is commonly associated with inherited bone marrow failure syndromes. CBCs obtained every 3–4 months have been recommended to assess for changes in the peripheral blood [174].

Ataxia Telangiectasia (ATM). Ataxia telangiectasia (A-T) is characterized by progressive ataxia and CNS degeneration, growth deficiency, ocular and facial telangiectasia, immunodeficiency, and an increased risk for malignancy. A-T is caused by pathogenic variants in *ATM* that hamper DNA repair with an incidence between 1 in 40,000 and 1 in 300,000 [178]. Diagnosis of A-T is made when two pathogenic variants in *ATM* are identified (homozygous or compound heterozygous) and/or when ATM kinase deficiency or absence is identified in the lymphoblastoid cell line (blood or fibroblasts) [179]. While life expectancy for individuals with A-T has increased over the years, it is still considerably decreased compared to average; lung disease (failure and infections) and malignancy are primary causes of mortality usually during or before the third decade of life. The overall incidence of malignancy in A-T patients ranges from 10% to 38%, with a particularly increased risk for lymphoid malignancies [116, 180, 181]. In a 2016 literature review of acute leukemia in A-T, 59 cases of hematologic malignancy were identified consisting of NHL ($N = 31$), T-cell ALL ($N = 18$), HL ($N = 5$), AML ($N = 3$), and B-cell ALL ($N = 2$) subtypes. Individuals with A-T have a 70-fold increase in leukemia risk, primarily ALL, with a four to fivefold increased risk in T-cell lymphoid malignancies in particular [182]. Individuals with A-T are very sensitive to ionizing radiation and cytotoxic chemotherapies. Tailored treatment plans should be considered when treating malignancies to reduce the risk for secondary malignancy and toxicity. A review of leukemia in A-T suggested that standard-of-care treatment will increase event-free survival (EFS) and toxicity, while reduced-intensity (modified) treatment will decrease EFS and increase quality of life. Further discussion about the impact of hematopoietic stem cell transplant (HSCT) in this population is needed. Lymphoid malignancies, specifically T-cell ALL and B-cell lymphoma, may present before age 5, which could be the presenting feature of A-T; thus, increased awareness of A-T is important for those treating pediatric lymphoid malignancies [181]. It has been suggested that genetic testing for A-T should be considered in any individual with a lymphoid malignancy and neurologic symptoms (or other feature of A-T) [183]. Patients with heterozygous ATM pathogenic variants remain at increased risk for cancer compared to the general population, primarily due to an increased risk for breast, prostate, and pancreatic cancers [180]; these solid tumors have widely recognized surveillance guidelines for early cancer detection which is an especially

important reason to identify A-T and offer testing to family members [177]. No formal hematologic surveillance has been recommended for patients with A-T, although parents are often advised to monitor for typical signs of malignancy including weight loss, bruising, and localized pain or swelling [178, 180]. Annual physical exam with CBC and complete metabolic profile can be considered, though there is little evidence to suggest that screening for leukemia improves survival [137].

Nijmegen Breakage Syndrome (NBN). Nijmegen breakage syndrome (NBS) results from pathogenic variants in the *NBN* gene (formerly *NBS1*), which codes for a protein product (nibrin) involved in DNA double-strand break repair in the same pathway as ATM [184]. Cytogenetically, cells from NBS patients show similar chromosomal breakage patterns to those from patients with A-T [185]. A founder mutation, c.657del5, is known to originate in Slavic populations in Eastern Europe and accounts for the majority of reported cases of NBS [186]. Individuals with NBS have distinctive dysmorphology, growth deficiency, immunodeficiency, cognitive impairment, and increased cancer risks [116, 186]. It is estimated individuals with NBS have a 40% risk to develop malignancy by age 20, with lymphoid malignancies being the most common [186–188]. Lymphoma is the most frequently identified malignancy in NBS, although 1/55 patients was reported to have ALL [187]. NBS is associated with unique types of diffuse large B-cell lymphoma and T-cell lymphoblastic lymphoma because of the presence of a clonal Ig/TCR rearrangement. Both ALL and AML have been reported in the NBS population as treatment-related malignancies [188]. The Polish Pediatric Leukemia and Lymphoma Study Group found that CNS involvement in leukemia was frequent in children with NBS [189]. Individuals with NBS are highly sensitive to radiation and chemotherapy treatments, and tailored treatment strategies should be considered. In a literature review from 2016, it was noted that 23% developed a secondary malignancy with 11 years as the median time between diagnoses [188]. Modified treatment protocols and the elimination of radiation have been shown to decrease toxic effects but not decrease risk for secondary malignancy [179]. Those with NBS may receive standard doses of chemotherapy, but their history must lack severe infections and immunoglobulin supplementation. HSCT may also be considered, especially in the event of secondary malignancy [188]. Those with a heterozygous pathogenic variant in *NBN* are thought to be at an increased risk for malignancy compared to the general population. This risk is thought to only be associated with the Slavic founder mutation (657del5) and associated with an increased risk for early-onset female breast cancer. Formal surveillance guidelines for early-onset cancer detection exist for women with this pathogenic variant, demonstrating another example of the benefits of identifying NBS early as it can lead to preemptive identification of other family members at risk through cascade testing [177]. Similar to A-T, patients with NBS should be monitored for general signs of malignancy, and parents should be informed of symptoms, but no formal hematologic screening guidelines are in place [186]. Consensus surveillance guidelines suggest annual CBC can be considered, especially in the event of hematologic malignancy symptoms [137].

Diamond-Blackfan Anemia (Over 20 genes reported, including *RPS19*, *RPS24*, *RPS17*, *RPL35A*, *RPL5*, *RPL11*, *RPS7*, *RPS26*, *and RPS10).* Diamond-Blackfan

anemia (DBA) is characterized by anemia (90% within the first year of life), short stature (30%), and congenital anomalies (50%) of the upper limb, craniofacial, heart, and genitourinary tract [190]. Anemia associated with DBA is characterized by reticulocytopenia, may be macrocytic or normocytic, and is often isolated without thrombocytopenia or neutropenia [191]. Pathogenic variants in genes encoding ribosomal proteins have been identified in approximately 50% of individuals meeting clinical diagnostic criteria, and the genetic etiology remains unknown for many cases. Pathogenic variants in *RPS19* account for approximately 25% of cases with an identifiable genetic cause, with the majority of DBA being associated with AD inheritance (~40% inherited from a parent) and a small portion associated with X-linked recessive inheritance [190, 192]. There is extreme variability of presentation, even within families, so some carrier parents may not come to attention until having a more severely affected child. DBA is associated with an increased risk for both AML and MDS. Risk analysis of the DBA registry (DBAR) found that the risk for AML was 5% by age 46 [193]. An increased risk was also observed for osteosarcoma, colon cancer, and female genital cancers, with a 20% cumulative risk for any type of malignancy by age 46 [193]. In an updated DBAR analysis of 702 patients from November 1991 to June 2016, 8 cases of MDS were reported with an actuarial risk (without competing factors) of 50% by age 30. There were three patients reported with AML [194]. Proposed leukemia surveillance includes complete blood counts (CBCs) several times a year with bone marrow aspirate/biopsy periodically or in the event of another cytopenia or a change in response to treatment [190, 195].

Shwachman-Diamond Syndrome (SBDS. Shwachman-Diamond syndrome (SDS) is characterized by hematologic abnormalities, pancreatic exocrine insufficiency, and skeletal abnormalities. This AR condition is caused by pathogenic variants in *SBDS* (homozygous pathogenic variants in *SBDS* are incompatible with life). Similarly, to the DBA-associated genes, *SBDS* also plays a role in ribosome biogenesis. In vitro studies have found that the SBDS protein co-localizes with the mitotic spindles, suggesting that spindle instability may contribute to bone marrow failure and leukemogenesis [196]. Neutropenia is the most common hematologic abnormality in those with SDS. A recent update of the SDS registry reported variable clinical presentations in 37 patients with SDS, such as neutropenia without steatorrhea ($N = 18$) [197]. SDS is associated with a significantly increased risk for hematological malignancies with a risk for malignant transformation reported between 5 and 36% [198]. The risk for AML has been reported to be 5–24%, with approximately one third of these cases preceded by MDS [199]. ALL and JMML have also been reported. A more recent study identified an approximately 9.8% risk to develop MDS/leukemia with a 24% risk to develop any hematologic abnormality (cytopenia and bone marrow failure included) [200]. In a study of patients with SDS, a total of 36 patients were included with MDS ($N = 26$) and AML ($N = 10$). Poor outcomes were identified in this cohort owing to high disease resistance and high treatment-related mortality [198]. Classic cytogenetic findings have been seen more frequently in SDS patients with myeloid malignancies, such as isochromosome of the long arm of chromosome 7 (44%), other abnormalities of chromosome 7 such as monosomy 7 (33%), and interstitial deletion of chromosome 20 (16%) [200]. It has been proposed that identification of those with SDS at a higher risk for leukemic

transformation could aid in treatment, specifically in the consideration of early HSCT [200]. Somatic acquisition of *TP53* has been proposed as a way to identify this higher-risk population; however, it is not yet clear how to incorporate this finding into the risk stratification [198]. Proposed leukemia surveillance includes CBCs every 3–6 months and bone marrow examination every 1–3 years or more frequently if clinically indicated [201]. The data for marrow surveillance in leukemia predisposition is sparse. A study showed that the 28% of patients with SDS were alive at 3 years in the group without marrow surveillance versus 62% in those with marrow surveillance. The authors suggested that compliance with other screenings may also be contributing to the improved outcomes [198].

Congenital Amegakaryocytic Thrombocytopenia (MPL). Individuals with congenital amegakaryocytic thrombocytopenia (CAMT) present with thrombocytopenia shortly after birth, typically without any congenital anomalies [202]. Progressive bone marrow failure is common, and approximately 68% will have pancytopenia or bone marrow hypocellularity by age 4 [203]. This condition is caused by recessive inheritance of pathogenic variants in the *MPL* gene, and while still rare, a founder mutation has resulted in a higher prevalence among the Ashkenazi Jewish population (1/22,500) [204]. In total, there have been less than 100 cases of CAMT reported in the literature [202]. *MPL* encodes the receptor for thrombopoietin, a growth factor that regulates the production and differentiation of megakaryocytes into platelets [205]. Genotype/phenotype correlations have been noted with variants that completely eliminate *MPL* activity being associated with a more severe presentation than variants that leave residual MPL function [103]. At this time, the risk for leukemia in CAMT is unclear because of small numbers. Only three reported cases of patients developing a pre-malignant condition or AML have been described, and only one of these patients had confirmed *MPL* pathogenic variants [203]. However, an accumulation of chromosomal abnormalities has been demonstrated as part of progressive bone marrow failure suggesting a theoretical possibility for malignant progression [203]. HSCT is the only curative option for CAMT, and it has been recommended to consider HSCT prior to development of pancytopenia [202, 203]. Outcomes of HSCT are better with reduced-intensity conditioning and matched related donors [206]. There are no recommendations for leukemia surveillance since the actual leukemia risk is unclear at this time.

Thrombocytopenia Absent Radii Syndrome (RBM8A). Thrombocytopenia absent radius (TAR) syndrome is characterized by thrombocytopenia and the hallmark feature of absent radii with the presence of thumbs [207]. Other congenital abnormalities including the skeletal, cardiac (tetralogy of Fallot and atrial septal defects among others), and genitourinary systems can occur. It should be noted that the absence of radii and presence of thumbs are physical distinctions that should be made for TAR syndrome, as opposed to FA where radial defects are also encountered but in the absence of thumbs. Cow's milk allergy is another common feature, and exposure may increase the severity of thrombocytopenia [207]. Unlike CAMT and many other progressive bone marrow failure syndromes, most children with TAR syndrome will have progressively fewer episodes of thrombocytopenia throughout childhood with eventual normalization, however may continue to have decreased platelet counts into adulthood [207, 208]. *RBM8A* inactivation seems to

be the underlying cause of TAR syndrome with biallelic loss of *RBM8A* through whole gene loss on one allele and a hypomorphic pathogenic variant of *RBM8A* in the other allele. Homozygous loss of *RBM8A* via null variants is thought to be incompatible with life. Most individuals with TAR have a confirmed deletion of 1q21.1, which includes several genes in addition to *RBM8A* [207]. *RBM8A* encodes a subunit of the exon-junction complex, which regulates mRNA translation and localization. In addition to the deletion of 1q21.1, the majority of affected children are heterozygous for one of two SNPs associated with reduced *RMB8A* expression [209]. Reports of leukemia in TAR syndrome are rare. Review of the literature identified five reports of AML in individuals with TAR syndrome, two in infants (2 months [210] and 1 year of age [211]), one in a 5-year-old [212], one in a 41-year-old patient [213], and one in a 47-year-old patient [214]. The 41-year-old adult patient had normal platelet levels prior to diagnosis, but she did develop severe thrombocytopenia during AML treatment [213]. The 47-year-old patient had varying platelet levels through childhood and adulthood, until the development of MDS with transformation to AML after 1 year of conservative treatment. HSCT is rarely utilized in the treatment of TAR syndrome [214, 215]. Platelet count is indicated in the setting of increased bleeding tendency, but no specific leukemia surveillance is recommended [216].

Severe Congenital Neutropenia (ELANE, G6PC3, GFI1, HAX1, CSF3R). Severe congenital neutropenia (SCN), also known as Kostmann syndrome, is characterized by defects in granulopoiesis and an increased risk for life-threatening infections. SCN is genetically heterogeneous, and several genes involved with the production and differentiation of the immune system play a role in this rare condition. AR (*HAX1*, *G6PC3*) and AD inheritance (*ELANE*, *GFI1*) can occur depending on the gene harboring the pathogenic variant(s) (*WAS* pathogenic variants also are associated with SCN, but are addressed separately in the section on Wiskott-Aldrich syndrome) [217, 218]. According to the SCN International Registry (SCNIR), pathogenic variants in the *ELANE* gene account for 45–60% of those registered [219, 220]. SCN-associated immunodeficiency is often treated with granulocyte colony-stimulating factor (G-CSF), which has greatly improved survival. However, prolonged use and higher doses of G-CSF have been associated with greater malignancy risk [218]. In a 2000 evaluation of 352 SCN patients treated with G-CSF from the SCNIR, it was found that 29 (8%) developed MDS or AML [221]. Other studies have found up to a 25% risk for AML [222, 223]. In a more recent study from the SCNIR, 374 patients were included in the analysis identifying a 22% risk to develop MDS/AML after 10 years. Data from this cohort showed the rate of MDS/AML development based on G-CSF dosing, citing that the risk for MDS/AML increased from 11 to 40% in those who were less responsive to G-CSF compared to more responsive patients [224]. The French Neutropenia Registry has also published data on leukemia development, citing 13 cases of leukemia in 231 patients with a cumulative incidence of MDS/AML of 2.7% by 10 years and 8.1% by 20 years [225]. It should be noted that the French cohort includes patients who have and have not received G-CSF compared to the SCNIR cohort who have all received G-CSF [219]. Kimmel and Corey have summarized the required accumulation of genetic variants required to progress from SCN to AML [226]. Individuals with SCN are born with

pathogenic variants in *ELANE*, *HAX1*, *G6PC3*, or *CSF3R*, and then an additional one to three somatic variants (*GCSF3R*, *ZC3H18*, *LLGL2*, or *RAS*) ± chromosomal loss or gain (monosomy 7, trisomy 21) are required for progression to MDS. After this, a total of one to nine somatic variants (*RUNX1*, *ASXL1*, *p300*, *CEBA*, *CSF3R*, *MGA*, *SUZ12*, *LAMB*, *FBXO18*, or *CCDC15)* ± chromosome loss or gain (monosomy 7, trisomy 21) are required for the final transformation to AML [226]. Of relevance, those with SCN due to pathogenic variants in *CSF3R*, *GCPC3*, or *TCIRG1* have little to no increased risk of MDS or AML development [227]. SCN-related MDS/AML requires HSCT for long-term cure. In a cohort of 136 SCN patients who underwent stem cell transplant following malignancy diagnosis, the 3-year overall survival was 82% with transplant-related mortality being 17%. Authors suggested that careful selection of HSCT candidates in the SCN population is important [228]. Patients with SCN that are not treated with HSCT will require close surveillance for malignant transformation to MDS/AML, although formal screening recommendations are lacking [229]. A recent review has included that the risk for transformation to MDS/AML can be managed with regular screening, including somatic variant analysis and karyotype analysis [219].

Telomere Biology Disorders (DKC1, *TERC*, *TERT*, *TINF2*, *NOP10*, *NHP2*, *WRAP53*, *RTEL1*, *CTC1*, *PARN*, *ACD*, *USB1).* Telomere biology disorders (TBD) result in significantly shortened telomeres compared to average due to pathogenic variants that disrupt the function of genes involved with regulating telomere maintenance [230]. To date, there have been 14 different genes described in this group of diseases, each associated with various phenotypes including age of clinical onset ranging from the neonatal period to the fifth and sixth decades of life [231]. The group of TBDs may result in specific physical findings, some which are classically associated with the well-known dyskeratosis congenita subtype. These sequelae include (1) dysplastic nails, (2) lacy reticular pigmentation (upper chest/neck), and (3) oral leukoplakia [230, 232]. Other findings in TBDs include increased risk for pulmonary fibrosis, bone marrow failure, malignancy, and liver disease [230, 232]. Of those that meet clinical diagnostic criteria for dyskeratosis congenita, only 70% will have an identifiable pathogenic variant [233]. There are various modes of inheritance for TBDs, such as X-linked (*DKC1*), autosomal recessive (*CTC1*, *NHP2*, *NOP10*, *PARN*, and *WRAP53*), autosomal dominant (*TERC* and *TINF2*), and autosomal dominant/recessive (*ACD*, *RTEL1*, and *TERT*). Genetic anticipation has been reported in the TBDs [234]. Diagnosis is generally made based upon physical exam findings and the identification of telomeres less than the first percentile (compared to age matched controls) through multi-color flow cytometry FISH on blood lymphocytes [235, 236]. Individuals with TBD are at high risk to develop MDS and AML, as well as other solid malignancies (head/neck cancers, anogenital cancers) and bone marrow failure [237]. The largest study to date on the incidence of malignancy in the TBD population from NCI's inherited bone marrow failure syndromes (IBMFS) cohort reported that there was a fourfold risk compared with the general population [238]. The risks for MDS and AML were reported at a 578- and 24-fold higher incidence compared to the general population, respectively. The risk for malignancy after transplant increased up to 30-fold higher [238]. Other studies have shown that individuals have an approximately 40% risk to develop malignancy by

age 50 [237]. The MDS incidence in DC is estimated to be between 3 and 33% by age 39, based on review of reported cases and the DC patients in the NCI's IBMFS cohort [237]. Another registry-based study estimated the cumulative risk for AML to be 10% by age 50 [175]. There are more therapy-related complications in those with TBD, so careful monitoring has been recommended [239]. There are no curative options for those with TBDs besides HSCT [231]. Guidelines for the management of hematologic manifestations in TBDs include baseline CBC with bone marrow aspirate/biopsy with careful morphologic examination and cytogenetic studies. Continued monitoring with annual CBC and bone marrow aspirate/biopsy is recommended if baseline exams were normal. Increased monitoring is recommended if abnormalities are detected [137, 231, 240].

11.9 Immunodeficiency Syndromes

WAS-Related Disorders (*WAS*). WAS-related disorders are a group of primary immunodeficiency syndromes ranging from severe to mild phenotypic spectrum and include Wiskott-Aldrich syndrome (WAS), X-linked thrombocytopenia (XLT), and X-linked congenital neutropenia (XLN). *WAS*-related disorders are inherited in an X-linked inheritance pattern caused by pathogenic variants in *WAS*. The diagnosis of individual syndromes within the *WAS*-related disorders is primarily based on clinical classification, although genetic testing can be helpful if a variant has been previously published. WAS classically presents with the triad of immunodeficiency, thrombocytopenia, and eczema. While inter- and intra-familial variability is common, most patients do suffer from thrombocytopenia and some degree of immunodeficiency [241]. Pathogenic variants which eliminate all WASP activity are generally associated with a more severe phenotype and deficiencies in multiple cell lineages, while variants that reduce (typically missense or splice site), but still leave, some residual function result in a milder, more variable phenotype [242, 243]. Individuals with some function and milder presentation predominately consisting of thrombocytopenia and no or minimal immune deficiency may be classified as XLT. Although a presentation of XLT may be manifested in childhood, additional more severe symptoms can present later in life resembling WAS. *WAS*-related disorders are not stagnant, and some prefer to discuss WAS as a spectrum from mild to severe rather than subdividing the diagnoses [241]. XLN is also caused by pathogenic variants in *WAS* and shares similar features to those who clinically appear to have WAS or XLT. However, unlike these, the primary cytopenia is neutropenia rather than thrombocytopenia. Interestingly, despite the presence of *WAS* pathogenic variants, there is normal WASP expression in XLN [244]. Four missense variants in exon 9 of *WAS* have shown to cause XLN [245]. All *WAS*-related disorders have an increased risk for malignancies. In retrospective studies of WAS cohorts, the risk for malignancy has been reported between 13 and 22% with a median age of 9.5 years [241]. The majority of malignancies diagnosed in this population are lymphoid, with additional reports of other leukemia. In a separate cohort of 301 WAS patients, 36 (12%) developed malignancy. The majority of cancers were

lymphoreticular tumors, and seven (2%) were leukemia (subtype unspecified) [246]. In another cohort of 194 WAS patients who underwent HSCT, 4 patients developed Epstein-Barr virus-related lymphoproliferative disease, and 1 developed JMML [247]. Recently, there have been additional reports of JMML-like disease in the *WAS*-related disorders [248, 249]. Kaposi sarcoma is a cancer typically associated with severe immunosuppression and HIV infection, but one patient has been reported with WAS that developed Kaposi sarcoma at age 24. This suggests that patients with WAS may also be at risk for a wider range of cancers associated with immunodeficiency [250]. In XLT, the risk for malignancy is not as high, as this milder phenotype manifests about a 5% risk for malignancy with the median age of diagnosis at 34 years of age. In XLN, males will present with congenital neutropenia associated with myelodysplasia, increased myeloid cell apoptosis, and lymphoid cell abnormalities [244]. Although a rare syndrome, 20–30% of males with XLN may be at risk for MDS and AML [251, 252]. HSCT is a curative option for the treatment of thrombocytopenia and immunodeficiency, especially for those with WAS [253]. Routine surveillance of CBCs has been recommended in WAS-related disorders, although clear guidelines for leukemia screening are lacking [244].

X-Linked Agammaglobulinemia (BTK). X-linked agammaglobulinemia (XLA), previously known as Bruton's agammaglobulinemia, is an X-linked primary immunodeficiency. This condition is caused by pathogenic variants in *BTK*, a gene required for B-cell maturation. Impaired B-cell differentiation and susceptibility to both viral and bacterial infections characterize this condition [254]. Other physical features have not been reported as part of this syndrome, but up to 35% may have short stature [255]. While hematological malignancy and solid tumors are increased in individuals with XLA, the risk remains ill-defined [256]. In a cohort from the Italian Primary Immunodeficiency registry, 164 males were included with XLA, and 3.7% were diagnosed with malignancy; none were hematologic malignancies [257]. In a cohort of 62 Chinese patients with XLA, one patient developed a T-cell lymphoma of the vocal cord at age 23, and the tumor rapidly progressed leading to death due to metastatic disease within 9 months [255]. In another cohort of 201 XLA patients, 4 developed malignancies including lymphoma without leukemia [258]. Despite the unknown risk for leukemia, a CBC with differential, chemistries, and quantitative serum immunoglobulins have been recommended at least once per year in patients with XLA [254].

11.10 Predisposition to Familial Leukemia

CEBPA Predisposition Syndrome (CEBPA). While somatic *CEBPA* variants occur in up to 10% of AML cases, some families have been identified with a germline *CEBPA* pathogenic variant predisposing to the development of AML [259–261]. *CEBPA* encodes for transcription factor CCAAT-enhancer-binding protein α, which is required for appropriate myeloid cell differentiation [260]. In families with germline *CEBPA* pathogenic variants, the *CEBPA* variant is often inherited in AD fashion with de novo cases reported in the literature [261]. It is thought that this

predisposition syndrome only causes an increased risk to develop AML without any congenital anomalies, antecedent cytopenia, nor myelodysplasia [262–264]. A recent study of 187 consecutive patients with AML identified 18 (10%) with *CEBPA* variants present in their leukemic cells; 2 of these 18 (11%) patients had a germline *CEBPA* variant identified, both of whom also had a first-degree relative with AML [265]. In another study where non-leukemic peripheral blood or buccal samples were available on 71 patients with *CEBPA* leukemic variants, 5 of these patients (7%) were found to harbor germline *CEBPA* pathogenic variants [266]. The germline *CEBPA* pathogenic variant is typically a frameshift variant and occurs in the N-terminal, while the somatically acquired variant occurs in the C-terminal. Few families with familial AML due to germline *CEBPA* pathogenic variant have been reported to date [259, 266]. The penetrance in families with germline *CEBPA* is nearly 100% [262]. The age of onset of AML in these families is variable with some individuals developing AML in childhood and their relatives not developing it until adulthood. However, in general, diagnosis typically occurs at a younger age than the average population [265]. In one study, average age of diagnosis was 24.5 years with a range of 1.75–46 years [262]. There has been a case of monozygotic twins with germline *CEBPA* variants who presented with AML diagnosed with a 13-year difference [267]. HSCT is often considered for AML treatment, though it should be noted that matched related donors should be tested for the germline *CEBPA* pathogenic variant before transplant due to adverse outcomes [264, 268]. Expert opinion guidelines exist for leukemia surveillance in germline *CEBPA*; these include baseline bone marrow biopsy/aspirate with annual CBC [269, 270].

RUNX1 Predisposition Syndrome (RUNX1). RUNX1 codes for a transcription factor involved in hematopoiesis [271]. Translocations and somatic single nucleotide variants involving *RUNX1* can be seen in the leukemic cells of patients with MDS and various types of leukemia, including AML, CMML, and ALL [271, 272]. Germline *RUNX1* pathogenic variants have been identified in families with thrombocytopenia, platelet dysfunction, and an increased risk for MDS and/or AML, which is transmitted in AD pattern [271]. This hereditary disorder is called familial platelet disorder with propensity to AML (FPD/AML). Pathogenic germline variants have been reported as missense, nonsense, splice site, indels, and copy number variants [273]. A recent analysis of *RUNX1*-mutated AML showed that ~30% (12/44) in this cohort were thought to have germline *RUNX1* pathogenic variants [274]. The risk of MDS/AML in those with germline RUNX1 variants is approximately 11–100%; recent estimates predict the average risk to be around 44% [275, 276]. The types of hematologic malignancies most frequently diagnosed are MDS and AML. T-cell ALL and B-cell ALL have been less frequently reported [275–277]. A study by Children's Oncology Group reported 13 germline *RUNX1* pathogenic variants in their cohort of 1231 cases of T-cell ALL [278]. Median age of leukemia onset is 33 years, although the age range can be quite broad (6–75 years) [271, 279]. Disease anticipation has been reported in those with germline *RUNX1* pathogenic variants [275, 276]. No other clear increased risks are present for solid malignancies in FPD/AML. The degree of thrombocytopenia can be normal to severe, though mild to moderate with an aspirin-like functional defect is average [277]. Platelets are typically normal in size, and functional platelet defects are often

what leads to excessive bleeding [276]. In situations where hematopoietic stem cell transplant is considered for treatment, genetic testing of matched related donors should be done for the familial RUNX1 variant due to risk for adverse outcomes, including poor engraftment and donor-derived leukemia [275, 277]. Similar to the other familial leukemia syndromes, consensus guidelines exist for leukemia surveillance in germline RUNX1, which include baseline bone marrow biopsy/aspirate with annual CBC [97]. Given the risk for thrombocytopenia, consideration of platelet aggregation studies is warranted [269, 270].

GATA2 Predisposition Syndrome (GATA2). *GATA2* codes for a transcription factor that is involved in hematopoiesis as well as vascular, urogenital, and neural development [280]. Initially, germline pathogenic variants in *GATA2* were thought to cause separate syndromes, such as familial MDS/AML, MonoMAC, and Emberger syndrome, due to wide phenotypic heterogeneity [275]. It is now known that there is significant overlap between these presentations; a disease spectrum ranging from mild to severe is more appropriate; thus, *GATA2* deficiency is a more applicable name for the syndrome [281]. The uniting denominator in *GATA2* deficiency is the risk to develop myeloid malignancy [282]. Germline variants are transmitted in an AD inheritance pattern, and there is significant variable expressivity between family members with the same *GATA2* pathogenic variant [281]. Presenting symptoms of patients with *GATA2* deficiency may range from immunodeficiency with hypocellular bone marrow failure and severe infections with warts to MDS without any pre-existing clinical features [282]. Severe onset of *GATA2* deficiency may look like MonoMAC syndrome, characterized by mycobacterial infections, monocytopenia, dendritic cell deficiency, NK cell and B-cell lymphocytopenia, and increased risk for MDS and AML [283]. Other severe manifestations may look like Emberger syndrome characterized by primary lymphedema with or without deafness and increased risk for MDS/AML [284]. There are growing reports of those with *GATA2* deficiency who present with MDS/AML without any clinical features [281]. In a cohort of 380 cases in the literature, average of myeloid malignancy onset was 19.7 years with a range between 12 and 35 years. Approximately 75% of those with *GATA2* deficiency developed myeloid malignancy [282]. Most patients develop MDS, and rarely de novo AML, with a high risk to transform to AML or CMML [282]. Important to mention is the frequency of *GATA2* deficiency in pediatric MDS. Over 600 cases of primary or secondary MDS were screened for *GATA2* deficiency, and it was identified that 15% of advanced MDS had *GATA2* deficiency, while 7% of all primary MDS had *GATA2* deficiency. Importantly, 72% of those with monosomy 7 and MDS in this cohort had underlying *GATA2* deficiency [285, 286]. There are no recommendations to consider hematopoietic stem cell transplant in those without overt malignancy in *GATA2* deficiency, but timely transplant has been recommended for those with malignancy owing to the increased transformation risks [282]. Genetic testing for the familial *GATA2* pathogenic variant should be performed on matched related donors prior to transplant to avoid adverse outcomes. Formal surveillance guidelines for *GATA2* deficiency do not exist. Consensus guidelines have been recently proposed and recommend annual CBC with a baseline bone marrow biopsy/aspirate [97]. Additional recommendations have been proposed for surveillance and management of other *GATA2* deficiency risks [270].

SAMD9 and SAMD9L Predisposition Syndromes (*SAMD9/L*). The *SAMD9/L* genes encode protein products involved in endosomal function and interferon signaling [287]. Germline pathogenic variants in these genes have recently been identified to cause multisystemic disorders with an increased risk for myeloid malignancy [288, 289]. Both disorders are associated with AD inheritance and GOF pathogenic variants [290]. Classically, pathogenic variants in *SAMD9* are associated with MIRAGE syndrome: myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy [289]. Classically, pathogenic variants in *SAMD9L* are associated with ataxia-pancytopenia syndrome [288]. The primary hematologic malignancy associated with these disorders is MDS with a subset of patients developing monosomy 7, though bone marrow failure has been recently reported [276, 291]. The loss of chromosome 7 has been proposed as a cellular adaptation to the growth-suppressive properties of the mutant SAMD9 or SAMD9L protein (also called adaptation by aneuploidy) [289]. A cohort of 46 cases of primary MDS were analyzed with 4 germline pathogenic variants in *SAMD9* and 4 germline pathogenic variants in *SAMD9L* identified. In this cohort, no additional systemic features such as ataxia-pancytopenia syndrome or MIRAGE syndrome were reported in the positive patients suggesting that isolated pediatric MDS may be a manifestation without additional symptoms [276, 287]. In another cohort of seven patients with germline pathogenic *SAMD9L* variants and MDS, age of diagnosis ranged from 1 to 42 years with a median of 2.1 years of age [292]. Another study of idiopathic, but suspected to be inherited, bone marrow failure patients used whole exome sequencing on skin fibroblasts to try to determine the underlying etiology of the marrow failure. In this cohort, 16 of the 179 patients were found to have germline pathogenic variants in *SAMD9/L*. Of those with germline pathogenic variants in *SAMD9L* (10 out of 16), all presented with severe BMF, five patients had monosomy 7, and only four had neurological symptoms. Of those with germline pathogenic variants in *SAMD9* (6 out of 16), all presented with mild BMF, five had monosomy 7, and only one had symptoms of MIRAGE syndrome [291]. More data is needed to determine risks and recommendations for hematopoietic stem cell transplant in this population of patients [293]. Genetic testing for the familial *SAMD9/L* pathogenic variant should be performed on matched related donors prior to transplant; blood or buccal samples may not be suitable specimen types due to the known mechanism of adaptation by aneuploidy [276, 294]. Expert-based recommendations have recently been published; CBC every 6–12 months is recommended with bone marrow biopsy/aspirate recommended at diagnosis and every other year if no hematologic symptoms/changes in CBC are noted. There are additional recommendations for other management of systemic manifestations of these disorders included as well [294].

ETV6 Predisposition Syndrome (ETV6). ETV6 is an ETS family transcription factor commonly involved in leukemia translocations (e.g., ETV6-RUNX1). The original publication described *ETV6* pathogenic variants in three separate kindreds with an AD history of thrombocytopenia and hematological malignancies [295]. Another team of investigators simultaneously described a different family with *ETV6* pathogenic variants and inherited thrombocytopenia, high erythrocyte mean

corpuscular volume (MCV), and 2 family members with B-cell ALL [296]; these same researchers then screened 23 additional pedigrees with thrombocytopenia and familial leukemia and identified 2 more inherited *ETV6* variants. As the original publications described, *ETV6* pathogenic variants are thought to confer an increased risk for thrombocytopenia and hematological malignancies of both myeloid and lymphoid lineages. The syndrome conferred by germline pathogenic variants in *ETV6* has been called thrombocytopenia 5. Thrombocytopenia is highly penetrant and is typically mild with mild bleeding symptoms and normal platelet size [297, 298]. Approximately 30% of those with pathogenic germline *ETV6* variants will develop hematological malignancy, most often B-cell ALL [299]. In families reported in the literature with pathogenic *ETV6* variants, 18 malignancies were reported in 72 patients with an additional 4 malignancies in cases without gene sequencing data available. Of the malignancies reported, 15 patients had B-cell ALL (typically pre-B-cell ALL) with a median age of 7 years at diagnosis. There were six myeloid malignancies reported, including AML, MDS, and CMML with a range of 8–82 years at diagnosis [297]. In a study of 4405 cases of pediatric ALL from the Children's Oncology Group and St. Jude Children's Research Hospital, 15 ETV6 variants were reported and predicted to be germline pathogenic variants. There was an overrepresentation of hyperdiploid karyotype in the germline cohort [300]. Solid tumors have been reported rarely, and the majority have been diagnosed in adulthood [297]. Genetic testing for the familial ETV6 pathogenic variants should be performed on matched related donors prior to transplant to avoid adverse outcomes. Consensus guidelines for hematological malignancy guidelines have been proposed and include regular CBCs with bone marrow biopsy/aspirates. Frequency of CBC and bone marrow biopsy/aspirates differ between guidelines [97, 299]. Additional information about management beyond leukemia risk also have been proposed [299].

PAX5 Predisposition Syndrome (PAX5). PAX5 variants are found in about 30% of pediatric ALL [301]. Through whole exome sequence analysis of a family with multiple cases of childhood ALL, a heterozygous germline variant, c.547G > A (p.Gly183Ser), was found in the paired box protein encoding gene, *PAX5* [302]. This germline pathogenic variant is one of the first genetic AD descriptions of pre-B-cell ALL susceptibility in a large kindred of childhood ALL. B-cell ALL cells from this family had monosomy 9p resulting from iso(9q). As *PAX5* is located at 9p13, loss of heterozygosity with retention of the mutant allele confirmed by Sanger sequencing is consistent with Knudson's two-hit hypothesis. Experiments in which the mutant construct was transfected into *PAX5*-null cell lines demonstrated partial loss of function compared to wild type, as measured by assays for two *PAX5* activation targets, CD19 and IgM expression. Drawing on over 20 ALL kindreds assembled by many collaborators, the same *PAX5* pathogenic variant was observed to co-segregate in another unrelated family also with multiple cases of ALL. Additional data, including RNA-Seq and expression array data, are consistent with the role of this *PAX5* germline pathogenic variant as a key determinant of B-cell ALL susceptibility. Interestingly, it seems to predispose to the subtype of B-cell ALL with 9p loss or isochromosome 9q. This is predisposition to B-cell ALL is newly described

and is thought to be rare. Surveillance recommendations are not yet available, although routine CBC monitoring and even bone marrow evaluation may be warranted in at-risk family members.

SH2B3 Predisposition Syndrome (SH2B3). A recent report describes germline homozygous *SH2B3* pathogenic variants in two siblings affected with developmental delay and autoimmunity, including one sibling who was diagnosed with B-cell ALL [303, 304]. *SH2B3* proteins are involved in many different signaling activities mediated by growth factor and cytokine receptors and play a critical role in the regulation of B lymphopoiesis, megakaryopoiesis, and expansion of hematopoietic stem cells [305]. In this described family with pathogenic *SH2B3* variants, the risk for leukemia predisposition appeared to be inherited in an AR pattern. *SH2B3* variants also have been associated with celiac disease type 13 and insulin-dependent diabetes mellitus [306, 307]. No formal surveillance recommendations have yet been offered for the management of rare patients diagnosed with pathogenic *SH2B3* variants.

IKZF1 Predisposition Syndrome (*IKZF1*). Somatic *IKZF1* variants encode the IKAROS protein, which is seen with increased frequency of B-cell ALL and is critical for lymphoid development [308]. In contrast to the N-terminal variants previously reported in a cohort of patients with primary immunodeficiency, germline pathogenic variants in *IKZF1* associated with increased risk for malignancy were identified beyond the N-terminus and zinc finger domains [309]. One family has been described in which six family members were identified to have a germline *IKZF1* pathogenic variant; two of the family members developed B-cell ALL [77]. In a cohort of newly diagnosed pediatric ALL patients by Children's Oncology Group and St. Jude Children's Research Hospital, approximately 43 germline *IKZF1* pathogenic variants were identified in 4963 patients [77]. In this cohort, all *IKZF1* variants were identified in B-cell ALL. It was also shown that the presence of a germline *IKZF1* pathogenic variant reduces response to therapy similar to somatic IKZF1 pathogenic variants [77]. The risk of leukemia for those with germline *IKZF1* pathogenic variants is unknown, but could be lower penetrance than expected [309]. There are no formal surveillance recommendations yet for those with germline *IKZF1* pathogenic variant.

11.11 Congenital Syndromes

Down Syndrome (Trisomy 21). Down syndrome (DS) is the most common aneuploidy syndrome, occurring in approximately 1 in 691 live births [116]. Most often it is caused by non-disjunction of chromosome 21 during gamete maturation resulting in trisomy 21. Only 3–4% of the time is DS the result of an unbalanced translocation involving chromosome 21, which can be inherited from a parent with a balanced translocation [310]. Individuals with trisomy 21 mosaicism also have been reported (approximately 1–2% of DS) [310, 311]. DS presents with distinct physical features, cognitive impairment, hypotonia, developmental delay, and an increased frequency of congenital heart defects (most often atrioventricular septal

defect), hearing loss, gastrointestinal malformations, polycythemia, TMD, and leukemia (ALL and AML, including acute megakaryoblastic leukemia [AMKL]) [116, 311]. A diagnosis of DS is typically made by a combination of dysmorphology examination and chromosomal analysis; many diagnoses of DS are now made prenatally due to available screening plus diagnostic amniocentesis or chorionic villus sampling.

Up to 10% of infants with DS will develop TMD [310–312]. An increased incidence of polycythemia (18–64%) is also seen in infants with DS [310]. The American Academy of Pediatrics recommends screening for TMD and polycythemia in patients with DS via CBC within 1 month of birth [310]. Among patients with TMD, 16–23% were found to develop AML [312–314]. An examination of the causes of death among patients with DS in Sweden (using data from three national registers) showed that leukemia was the reported cause of death in 2.2% of patients with DS between 1969 and 2003 [315]. Children with DS have a 10- to 20-fold increased risk of ALL or AML, and an up to 500-fold risk of AMKL, compared with non-DS children [316, 317]. Increased toxicity to standard AML and ALL treatments has been observed in patients with DS, necessitating tailored treatment approaches [311, 318]. Interestingly, individuals with DS appear to have a lower incidence of solid malignancies than individuals without DS; the reason for this has not yet been elucidated [315].

Other Congenital Disorders. Childhood leukemia also has been described in association with other rare congenital conditions or birth defects [319–321]. Most of the specific leukemia-associated birth defects are based on a handful of case reports, and so it is difficult to assess the actual risk for leukemia and any required screening procedures. Some of the other non-DS leukemia-associated congenital disorders include Goldenhar's syndrome [322], Rubinstein-Taybi syndrome [323], Treacher Collins syndrome [324], Poland's anomaly [324], Klippel-Feil syndrome [325], and hypomelanosis of Ito [326]. As next-generation sequencing becomes more common, more leukemia-associated congenital disorders with specific variants contributing to leukemia risk may be discovered.

11.12 Conclusions

Since the identification of the first families with hereditary predisposition to leukemia, knowledge of the germline genomic landscape has rapidly expanded to include many genes associated with syndromic and non-syndromic predisposition syndromes. Until recently it was thought that predisposition to childhood leukemia was extraordinarily rare; however, the field of cancer predisposition now recognizes the genetic risk for leukemia is more common than previously realized [309, 327]. Although understanding of leukemia predisposition has grown, there still remains much to learn including better-defined genetic testing guidelines, improved estimates of penetrance for newly identified pathogenic variants, optimal surveillance guidelines for affected individuals, and implications for treatment [97, 328, 329] (Table 11.1).

Table 11.1 Details of Hereditary Pediatric Leukemia Syndromes, including screening recommendations when available

Category	Syndrome	Gene(s)	Inheritance	Prevalence	Leukemia type	Guidelines available for leukemia screening recommendations
DNA repair syndromes	Li-Fraumeni syndrome	*TP53*	AD	1/5000–1/20,000	ALL, MDS, AML	Yes [96]. No specific recommendations made for leukemia; counsel for symptom awareness
	Constitutional mismatch repair deficiency syndrome	*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*	AR	1/1,000,000	T-cell ALL, AML	Yes [109, 112]. CBC every 6 months starting at 1 year of age
RASopathies	Neurofibromatosis type 1 (NF1)	*NF1*	AD	1:3000	CMML/JMML, AML	Yes [149, 150]. Counsel for symptom awareness. The presence of juvenile xanthogranulomas should raise suspicion to assess for JMML
	CBL syndrome	*CBL*	AD	Unknown; 3/65 patients with JMML were found to have germline CBL variants [159]	JMML	Yes [156]. Physical examination with spleen evaluation and CBC every 3–6 months
	Noonan syndrome	PTPN11	AD	1/1000–1/2500	TMD, JMML, CMML, ALL	Yes [156]. Specific PTPN11 and KRAS pathogenic variants would benefit from physical examination with spleen evaluation and CBC every 3–6 months

Bone marrow failure syndromes	Fanconi anemia	Over 16 genes, including FANCA	AR, XL	Unknown; carrier frequency in N. America is 1/181 and in Israel 1/93 [330]	AML, MDS, ALL	Yes [174]. Annual bone marrow biopsy/aspirate starting at age 2; CBCs every 3–4 months
	Ataxia telangiectasia	*ATM*	AR	1/40,000–1/100,000	ALL	Yes [137]. Annual physical exam with CBC can be considered
	Nijmegen breakage syndrome	*NBS1*	AR	1/100,000; carrier frequency of founder mutation approximately 1/155 (Eastern Europe, Bavaria)–1/34 (Sorbians) [186, 331]	ALL, TLBL/ALL (Nijmegen study group)	Yes [137]. Annual CBC can be considered
	Diamond-Blackfan anemia	*Over 20 genes, including RPS19*	Majority of cases are de novo, but AD inheritance also occurs. XL inheritance has been reported in *GATA1* family	1/200,000–1/100,000 [195]	MDS/AML, ALL	Yes [195]. CBC several times a year with bone marrow biopsy/aspirate periodically
	Shwachman-Diamond syndrome	*SBDS*	AR	1/76,000 [332]	MDS/AML, ALL	Yes [201]. CBC every 3–6 months with bone marrow examination every 1–3 years
	Congenital amegakaryocytic thrombocytopenia	*MPL*	AR	1/22,500 in the Jewish population [333]; rare in other populations	MDS/AML	None

(continued)

Table 11.1 continued

Category	Syndrome	Gene(s)	Inheritance	Prevalence	Leukemia type	Guidelines available for leukemia screening recommendations
	Thrombocytopenia and absent radii (TAR) syndrome	*RBM8A* *Del 1q21.1*	AR, approximately 25% of cases have a de novo 1q21.1 deletion on one allele	1/200,000–1/100,000 [207]	MDS/AML [211–213]	None
	Severe congenital neutropenia/Kostmann	*ELANE* *G6PC3* *GFI1* *HAX1* *CSF3R*	Depending on the gene involved inheritance may be AD or AR	15/1,000,000–10/1,000,000 [218]		Yes [219]. Regular screening with somatic variant analysis and karyotype analysis
	Telomere biology disorders	*CTC1* *DKC1* *TERC* *TERT* *TINF2* *NOP10* *NHP2* *WRAP53*	Predominately XL, but AD and AR are possible depending on the causative gene [232]	Rare; prevalence is unknown [232]	MDS/AML	Yes [240]. Baseline bone marrow biopsy/aspirate with CBC, regular follow-up recommended
Immunodeficiency	WAS-related disorders	*WAS*	XR	4/1,000,000 males [246]	ALL	None
	X-linked agammaglobulinemia	*BTK*	XL	6/1,000,000 [254]	ALL	Yes [250]. CBC with differential recommended annually

Genetic Instability/ RECQ helicase deficiency	Werner syndrome	*WRN*	AR	1/20,000–1/40,000 in Japan (founder mutation); 1/200,000 in the United States [334]	AML, MDS	None
	Rothmund-Thomson	*RECQL4*	AR	Rare, exact incidence unknown	MDS	None
	Bloom syndrome	*BLM*	AR	1/100 carrier frequency in Ashkenazi Jewish population	AML, ALL, MDS	Yes [124]. Counsel for symptom awareness
Immunodeficiency	WAS-related disorders	*WAS*	XR	4/1,000,000 males [246]	ALL	None
	X-linked agammaglobulinemia	*BTK*	XL	6/1,000,000 [254]	ALL	Yes [250]. CBC with differential recommended annually
Predisposition to familial leukemia	CEBPA predisposition syndrome	*CEBPA*	AD	Unknown, rare	MDS, AML	Yes [97]. Baseline bone marrow biopsy/aspirate with annual CBC
	RUNX1 predisposition syndrome	*RUNX1*	AD	Unknown, rare	MDS, AML	Yes [97]. Baseline bone marrow biopsy/aspirate with annual CBC
	GATA2 predisposition syndrome	*GATA2*	AD	Unknown, rare; approximately 25 families have been reported [335]	MDS, AML	Yes [97]. Baseline bone marrow biopsy/aspirate with annual CBC
	SAMD9/L predisposition syndrome	*SAMD9*, *SAMD9L*	AD	Unknown, rare	MDS	Yes [294]. Bone marrow biopsy/aspirate at diagnosis with CBC annually. Consider follow-up bone marrow evaluation every other year

(continued)

Table 11.1 continued

Category	Syndrome	Gene(s)	Inheritance	Prevalence	Leukemia type	Guidelines available for leukemia screening recommendations
	ETV6 predisposition syndrome	*ETV6*	AD	Unknown, rare	ALL	Yes [97, 299]. Baseline bone marrow biopsy/ aspirate with annual CBC. Frequency of screenings differs between guidelines
	PAX5 predisposition syndrome	*PAX5*	AD	Unknown, rare	ALL	None
	SH2B3 predisposition syndrome	*SH2B3*	AR	Unknown, rare	ALL	None
	IKZF1 predisposition syndrome	*IKZF1*	AR	Unknown, rare	ALL	None
Aneuploidy	Down syndrome	Trisomy 21	Sporadic (aneuploid) (94%), mosaic (2.4%) unbalanced translocation (3.3%) [116]	1/660	TMD, ALL, AML (AMKL)	Yes [310]. CBC 1 month after birth.

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Chapter 12
Inherited Pediatric Cancer in Low- and Intermediate-Resource Countries

Maria Isabel Achatz, Patricia Ashton Prolla, Hany Ariffin, and Pierre Hainaut

Abstract Childhood cancer statistics are scarce and incomplete in most emerging and low-income countries (ELIC), in which up to 75% of the cases may arise. In many low-resource contexts, lack of awareness, scarce diagnostic resources, and poor access to care are serious barriers to the recognition of inherited pediatric cancer. Family history is rarely investigated in a systematic way, and genetic testing is usually limited or absent in most ELIC. Therefore, the burden of inherited childhood cancer in low-resource countries is poorly known and is most likely underestimated. Available data reveal important differences in the clinical patterns of inherited childhood cancer between high- and low-income regions. These differences are largely explained by biases in detection, diagnosis, reporting, and management due to constrained socioeconomic resources. Only a small number of national cancer institutions in ELIC have formulated coordinated programs toward inherited childhood cancer. In this chapter, we describe leading initiatives in two intermediate-resource countries representative of large populations in transitions: Malaysia and Brazil. We also discuss the identification in defined population groups of germline mutations due to founder effects, which can lead to large clusters of inherited childhood cancers. Finally, we provide a brief overview of the patterns of retinoblastoma and nephroblastoma in the low-income settings of sub-Saharan

M. I. Achatz
Cancer Genetics Unit, Centro de Oncologia, Hospital Sirio-Libanês, São Paulo, Brazil

P. A. Prolla
Department of Genetics, Federal University of Rio Grande do Sul and Oncogenetics Program, Hospital de Clínicas de Porto Alegre, (HCPA), Porto Alegre, Brazil

H. Ariffin
Department of Pediatrics, University of Malaya, Kuala Lumpur, Malaysia
e-mail: hany@ummc.edu.my

P. Hainaut (✉)
Institute for Advanced Biosciences, Inserm 1209 CNRS 5309 University Grenoble Alpes, Grenoble, France
e-mail: pierre.hainaut@univ-grenoble-alpes.fr

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Africa, and we discuss the plausible contribution of germline mutations to cancer burden in these areas.

Keywords Low-income countries · Socioeconomic resources · Geographic incidence · Epidemiology

12.1 Introduction

About 5–10% of pediatric cancers are associated with genetic predisposition [1]. A comprehensive analysis of the genetic landscape in a cohort of 981 pediatric cancer cases of Caucasian ethnicity has identified an association with a pathogenic germline variant in 7.6% of the cases. Most pediatric germline variants are associated with dsDNA repair (*TP53, CHEK2, MSH2, MSH6, BRCA2*), transcription control (*TP53, VHL, RB, LZTR1*), or epigenetic reprogramming. Pediatric cancers most commonly associated with pathogenic germline variants include adrenocortical carcinomas, nephroblastomas (Wilms tumors, WT), retinoblastomas (RB) (>50%), hypodiploid B-ALL, specific molecular subtypes of gliomas (K27wt), atypical teratoid rhabdoid tumors, and medulloblastomas carrying SSH mutations (10–50%) [2].

According to Cancer Today, the total number of cancers in 2018 was 200,166 in the age group 0–14 years [3]. The worldwide age-standardized rate (ASR) was 10.2 per 10^5 person-years, with significant variations among countries and world regions. When considering a division of the world's populations based on the Human Development Index (HDI) of the United Nations Development Programme (UNDP), 104,510 cases (52%) occurred in countries with low or intermediate HDI (HDI < 0.800) [4]. The highest estimates were reported for Western Europe and North America (ASR: 18.2 and 15, respectively). The 20 countries with estimated ASR below 5.0 person-years included 10 countries in sub-Saharan Africa, 5 in the Caribbean, 3 in the Pacific Islands, 1 in the Middle East, and 1 in Central Asia.

Types of frequent childhood cancers show striking differences in relation with HDI (Fig. 12.1). Data from Cancer Today show that leukemia and CNS tumors are the most frequent neoplasms in high and very high HDI countries [2]. These cancers show a decreasing trend of incidence from very high HDI countries (ASR: leukemia 5.2, CNS tumors 1.7) to low HDI countries (leukemia 1.2, CNS tumors 0.46). This trend may reflect poor access to diagnostic tests in low HDI countries. In contrast, the cancers ranking third (non-Hodgkin lymphoma, ASR: 1.0) and fourth (kidney, ASR: 0.92) in very high HDI countries were equally frequent in low HDI countries (ASR 1.2 and 0.91, respectively, ranking first and second in these countries). This concordance may be due to the fact that these cancers are primarily diagnosed on overt clinical symptoms and therefore less dependent upon cost-intensive resources.

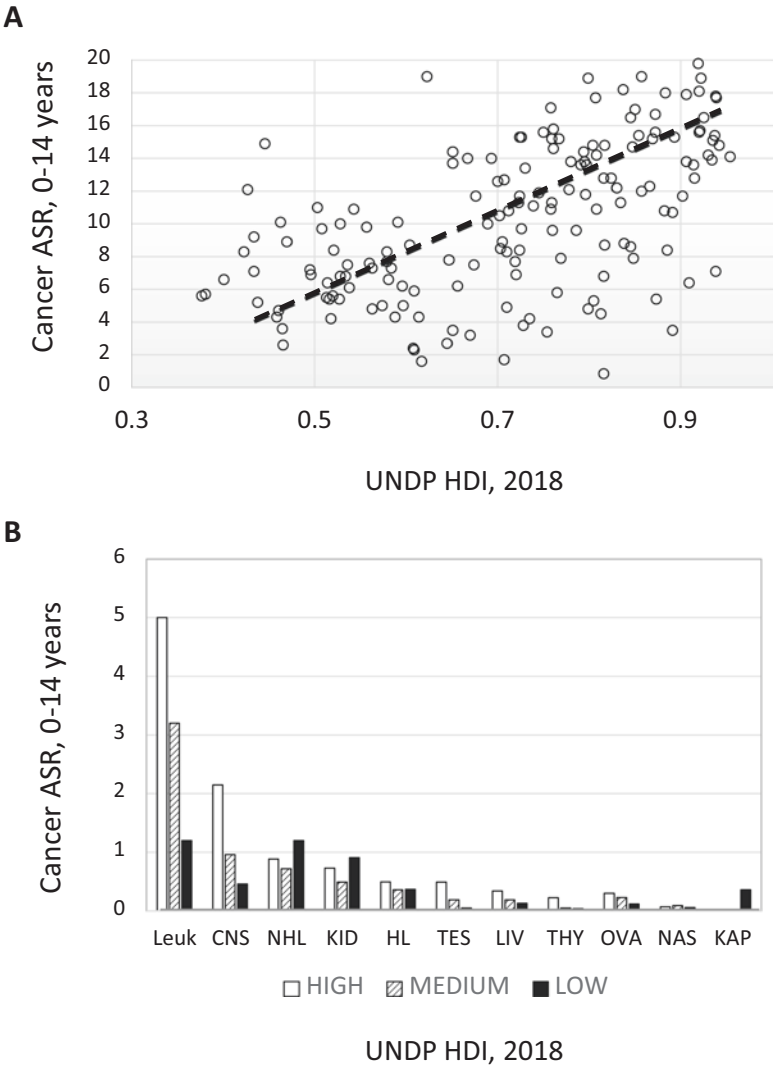

Fig. 12.1 Correlation between incidence rates of pediatric cancers (0–14 years) and Human Development Index in 135 countries. Estimates of incidence rates (ASR, age-standardized rates) from Cancer Today [3] were plotted against the 2018 Human Development Index of the United Nations Development Programme for 135 countries and territories of more than one million inhabitants

12.2 Geographic Variations of Inherited Pediatric Cancers

Due to lack of data, there is little evidence that the burden of genetic predisposition to pediatric cancer strongly varies across regions. Among cancers frequently associated with predisposition, RB and WT are perhaps the most consistently ascertained globally because they produce clear clinical signs and therefore can be diagnosed without the support of advanced technologies. Their rates of incidence appear remarkably uniform across the world, with similar age distribution and histopathological features, independent of world regions and ethnicity. This observation suggests that the population rate of occurrence of underlying germline mutations is relatively constant across ethnicities. Nevertheless, differences may exist, and studies in Kenya have pointed to an overrepresentation of certain ethnic groups among patients treated for WT, suggesting increased incidence within specific populations as well as different survival rates [5]. For both RB and WT, the most significant differences across populations are age at presentation, stage at diagnosis, and outcome, which are late, advanced, and dramatically poor in most low-resource settings. These differences are most likely caused by lack of resources rather than genetic or biological differences in the characteristics of these cancers.

Geographic variations and differences have been reported in relation with the population prevalence of founder variants. A founder variant is a genetic alteration observed with high frequency in a group that is, or was, geographically or culturally isolated, in which one or more of the ancestors were carriers of the altered gene. Examples of inherited childhood cancer syndromes with strong founder variant effects include *xeroderma pigmentosum* (XP), a clinically and genetically heterogeneous genodermatosis characterized by cutaneous and eye hypersensitivity to ultraviolet-induced changes and high risk of skin cancers. XP patients carry germline mutations in one of the seven genes encoding proteins of the nucleotide excision repair (NER) pathway or in POLH, encoding the translesion synthesis polymerase eta [6]. The disease has an estimated prevalence of 1–2.3 cases per million live births in the USA and Western Europe, respectively, whereas estimates from the 1970s suggested an incidence of 1 in 20,000 live births in Japan. More than half of the Japanese XP patients are homozygous for the same founder variant in the XPA gene [7]. A founder mutation in the XPC gene is responsible for the highest ever reported prevalence (1 in 5000 live births), observed in the Comorian population of Mayotte. This mutation was found to be of African origin with an estimated age of about 770 years, suggesting that the same mutation may be responsible for cases of XP on the African continent [8]. Another founder XPC mutation is detected in 74% of the XP cases in Northern Africa and associated with a high degree of consanguinity [9]. The same XPC founder variant from the Comorian population was identified in a Brazilian cohort of XP patients. However, the relationship between Brazilian and Mayotte populations carrying the same variant was not

investigated. A plausible explanation is that the Portuguese slave traders on the east coast of Africa, mostly Mozambique, may have brought individuals who carried the XPC variant to Brazil [10]. Thus, differences in prevalence and in patterns of the disease occur as the consequence of founder mutations exhibiting specific genotype/phenotype correlations and stabilized by consanguinity. The genetic variations associated with ethnicity of the carrier's genome do not appear to exert a significant modifier effect on penetrance or clinical patterns.

Mutation of the *TP53* suppressor gene is the most common genetic event associated with childhood cancer. In a cohort of 981 pediatric cancers, germline *TP53* mutations were identified in 25 cases (2.6%). The most common clinical form of cancer predisposition associated with *TP53* mutations is the Li-Fraumeni syndrome (LFS), characterized by the early occurrence of adrenocortical tumors (ACT), brain tumors (with a high proportion of choroid plexus carcinomas (CPT) and medulloblastomas (MED)), and soft tissue sarcoma (STS), including a high proportion of embryonal rhabdomyosarcoma (RMS), osteosarcoma, and hematopoietic malignancies [11]. The IARC *TP53* database of germline mutations contains information on pediatric cancer patterns in families from Northern America, Western Europe, and Asia, making it possible to compare pediatric tumor patterns between these populations (Fig. 12.2). LFS in South America has been extensively studied in relation to the Brazilian founder mutation and will be described later in this chapter.

The types and location of mutations along *TP53* are extremely similar across the three population groups. The mean age at diagnosis is between 4.5 and 6.5 years with no significant difference among populations. Accrual with age shows a nonsignificant tendency for cancers to be diagnosed at earlier ages in the Northern American group compared to Western European and Asian groups. The main types of childhood cancers are the same in the three groups. However, the proportion of different diagnoses shows borderline significant variations among populations (unadjusted p value = 0.029). The highest proportion of ACT is observed in Asians (24.2%) and the lowest in Western Europeans (8.1%). In contrast, Western Europeans showed a higher proportion of CNS tumors (44.6%) than North Americans (34.5%) and Asians (24.2%). Sarcomas are equally represented in the three population groups, but the proportion of sarcomas identified as RMS varies considerably, with a higher proportion in Western Europe and Asia than in Northern America. Thus, despite the fact that the clinical consequences of carrying a germline *TP53* mutation are remarkably uniform in the three population groups, the frequency at which each particular form of LFS childhood cancer occurs may differ according to population and/or ethnicity. Further studies are needed to determine whether these differences are due to ethnicity-related genetic polymorphisms acting as modifiers of *TP53* mutation penetrance, to differences in exogenous and lifestyle risk factors, or to biases in the diagnosis of childhood cancers and in the detection of LFS/LFL families.

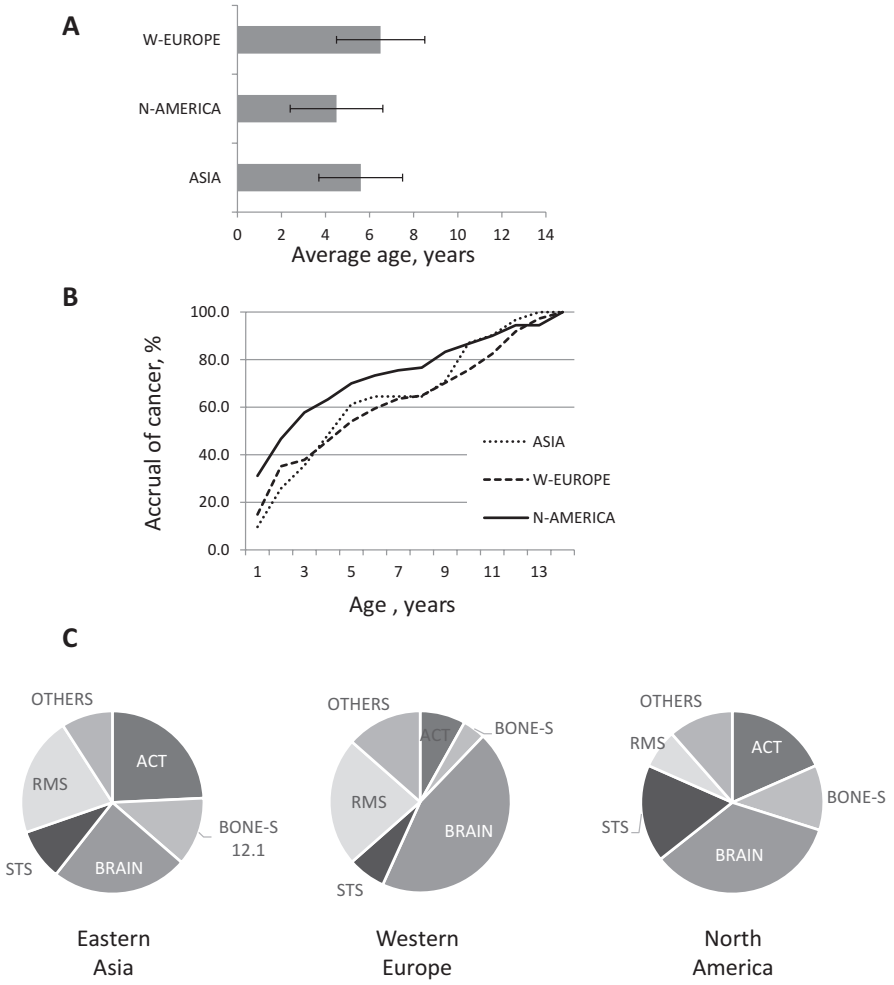

Fig. 12.2 Pediatric cancers in carriers of germline *TP53* mutations in three populations. Data on pediatric cancer (diagnosed at <15 years) were compiled for selected population groups in the IARC germline *TP53* mutation database (http://p53.iarc.fr/TP53GermlineMutations.aspx). The dataset included 33 cases from Asia (Taipei (2), Japan (16), Korea (3), Malaysia (11), and Singapore (1)), 74 cases from continental Western Europe (Austria (6), France (39), Germany (15), Switzerland (5), and The Netherlands (9)), and 115 cases from Northern America (Canada (23), USA (90), not specified (2)). (**a**) Average age (± SD) at diagnosis. (**b**) accrual of cancer diagnoses with age (adjusted at 100% at 15 years in each population). (**c**) Proportion of different cancer diagnoses (chi square $p = 0.029$ for the comparison between the three population groups). *ACT* adrenocortical tumor; *BONES-S* osteosarcoma; *BRAIN* brain tumors; *STS* soft tissue sarcoma; *RMS* rhabdomyosarcoma; *OTHERS* all other childhood cancer diagnoses

12.3 Inherited Pediatric Cancer in Malaysia

Malaysia is a 329,847-square kilometer country essentially consisting of two large territories, Peninsular Malaysia and Malaysian Borneo, as well as of many smaller islands. It represents the most southeastern point of the Eurasian continent. Its multiethnic, multicultural population of 31.6 million (mid-year 2018 estimate) comprises ethnic Malays (50.4%), Chinese (23.7%), Indians (7.1%, mostly of Tamil community), and indigenous ethnic groups (Thais, Khmers, Chams, and natives of Sabah and Sarawak). The HDI is 0.804 (2018; world rank: 61/180), and the nominal GDP per capita is USD 11,378 (2018 Word Bank estimates). About 29% of the population is aged 0–14 years. According to Cancer Today, the estimated ASR for childhood cancer (0–14 years) is 9.6/10^5 person-years in 2018 [3]. The most common pediatric cancers are leukemia (39.8%), CNS tumors (12.3%), non-Hodgkin lymphoma (6.9%), and kidney cancers (4.3%). Over the past 20 years, pediatric oncology in Malaysia has focused on the introduction of effective treatment strategies for childhood leukemia's and on assessing differences in treatment responses in relation to ethnic diversity [12]. Currently, the event-free survival rates of children with acute lymphoblastic leukemia in Malaysia are comparable to results in the West [13].

A program for detecting and managing families with high risk of childhood cancer has been developed at the Department of Pediatrics, University of Malaya Medical Centre (UMMC), Kuala Lumpur, the largest university-based pediatric oncology unit in the country. This initiative has led to increased awareness at the national level, leading to the development of a network of institutions across the country. The first Malaysian kindred with Li-Fraumeni syndrome was identified in 2007, where the proband was a girl who developed rhabdomyosarcoma in infancy and suffered a brain recurrence at the age of 8 years [14] (Fig. 12.3). An in-house

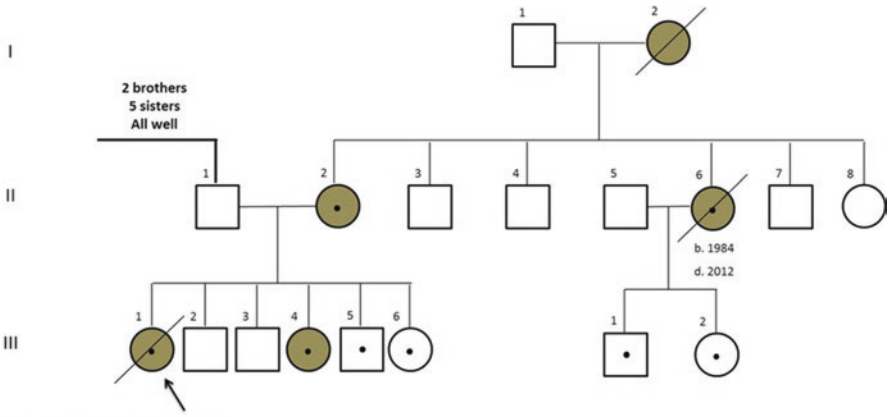

Fig. 12.3 Pedigree of the first Malaysian kindred identified with Li-Fraumeni syndrome. (From [14])

laboratory was set up for *TP53* mutation analysis with duplicate samples sent for confirmatory studies to a reference laboratory (IARC, Lyon, France). Counseling and follow-up were done on an ad hoc basis. Blood tests, imaging, and treatment were performed according to the standard protocols for each individual cancer.

A nationwide program to identify families with LFS was started in 2010 under the auspices of the Malaysian Society of Paediatric Haematology and Oncology involving pediatric oncologists throughout the country. Using childhood adrenocortical carcinoma (ACC) as the sentinel cancer, irrespective of familial cancer history, patients from various hospitals in Malaysia were recruited into a pilot study. Using the national network of pediatric oncologists, shared facilities were developed for standard procedures of counseling, obtaining consent and disease surveillance as well as a central laboratory for genetic testing. In a 2-year study period, three of four children with ACC were found to carry *TP53* mutations and showed familial cancer patterns, and cascade *TP53* testing detected mutations in relatives of two probands [15].

A surveillance program has been established, inspired by surveillance recommendations developed for Western patients [16]. Unaffected carrier children are followed-up twice yearly in the pediatric oncology outpatient clinic. Adult carriers are being followed-up in an adult risk management clinic, with a focus on the risk of inherited breast cancer. However, constraints, namely, cost and local availability, determine the choice of surveillance investigations for unaffected carriers. For instance, the surveillance strategy for imaging is limited to ultrasonography of the abdomen in children, while expensive modalities, such as magnetic resonance imaging or positron emission tomography, are only prescribed if there are suggestive symptoms or signs of cancer. The current strategy for screening and managing *TP53* mutation carriers using the pediatric oncology consortium in Malaysia is shown in Fig. 12.4.

In emerging countries without a structured oncogenetics service, it may be practical to use a nationwide group of pediatric oncologists to detect inherited cancers. Indeed, in most countries, pediatric oncologists are part of a national network or society providing a basis for developing common practice. In contrast, LFS patients presenting with cancers in adulthood are more likely to be managed by physicians or surgeons according to anatomical site, and, in this context, familial history may be easily overlooked due to lack of awareness by both patients and doctors. Typical of such a situation, one Malay family was only recognized to have LFS when the proband presented to the pediatric oncologist with ACC at age 4 years, despite the previous diagnosis of osteosarcoma at age 16 years in a paternal uncle (managed by an orthopedic surgeon) and of breast cancer at age 38 years in a paternal aunt (managed by breast surgeon).

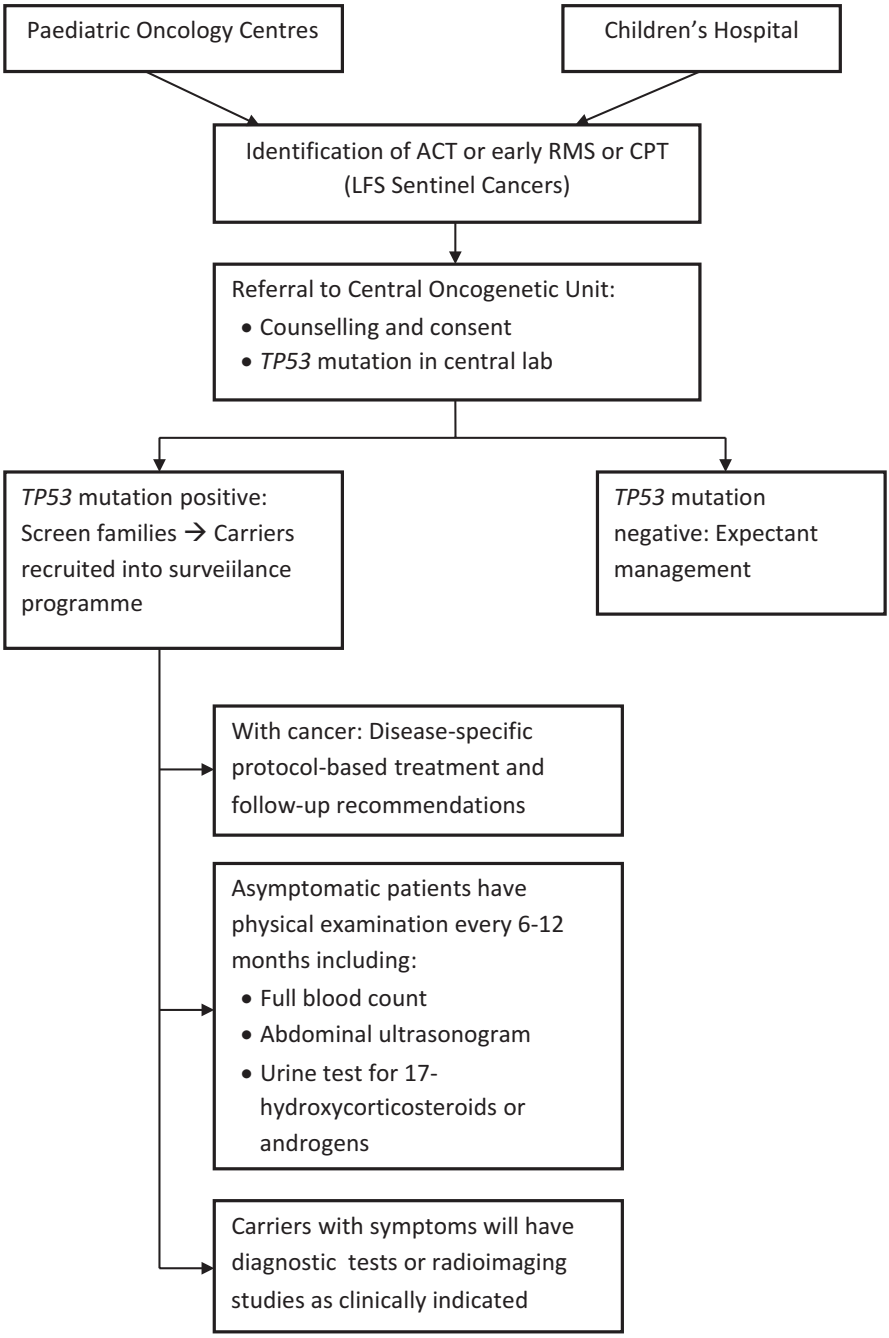

Fig. 12.4 Strategy for screening and managing TP53 mutation carriers in pediatric oncology practice at the University of Malaysia, Kuala Lumpur. *ACT* adrenocortical tumor; *CPT* choroid plexus tumor; *RMS* rhabdomyosarcoma

12.4 Inherited Pediatric Cancer in Brazil

Brazil is the largest country in Latin America (8,515,762 square kilometers). Its total population of over 200 million is quite diverse with many Brazilians having multi-ethnic ancestry, with a predominance of Mediterranean and northern European as well as African and, to a lesser degree, Asian and Amerindian contributions. However, when genetic markers of ancestry are studied, there is a predominance of European alleles (ranging from 60 to 80%) in all five regions of the country [17]. The HDI is 0.761 (2018, world ranking: 79/180), and the nominal GDP per capita is USD 8921 (2018 World Bank estimates). Brazil shows very large socioeconomic disparities between North and South, as well as between rich and poor areas in each of its five main regions. The Gini coefficient, a measure of economic disparity, is one of the highest in the world (2018: 53.9). According to Cancer Today, the estimated ASR for childhood cancer (0–14 years) was 14.9/10^5 person-years in 2018, with the most common diagnoses being leukemia (31.0%), brain cancer (13.0%), non-Hodgkin lymphoma (7.0%), and testicular cancer (5.6%) [3]. However, incidence rates and types of cancer are very heterogeneous from one region to the other. A survey of 14 population-based cancer registries across the country has reported incidence rates (0–14 years) ranging from 9.4 (Salvador) to 22.6 (Goiania) per 10^5 person-years, with a tendency for consistently high incidence rates in the more densely populated areas of Southern Brazil (Porto Alegre, 18.8; Curitiba, 18.8; Sao Paulo, 18.6 per 10^5 person-years) [18]. High incidences of childhood osteosarcoma and WT tumors are observed in several registries (Sao Paulo, Goiania), whereas consistent reports have indicated that the incidence of childhood adrenocortical carcinoma (ACC) in Sao Paulo, at 1.5–2.0 per million person-years, was the highest reported in the world (three to ten times the average rates of high-resource countries) [19].

12.4.1 Two Decades of Building Oancogenetics in Brazil

The development of pediatric oncogenetics in Brazil over the past 25 years provides an interesting model for setting up comprehensive strategies in other emerging countries. In the mid-1990s, several centers initiated familial cancer risk evaluation clinics focused on the diagnosis and management of patients and families with hereditary cancer syndromes including pediatric cancers. The impetus came from the most clinically advanced centers in large cities of Southeast Brazil and was supported by research programs that provided for the build-up of expertise in genetic testing. The rapid development of public awareness for familial cancer, in a context of economic growth and increased public and private spending in public health, has boosted efforts for building a national network in cancer genetics, encompassing inherited pediatric cancers. A national network of familial cancer genetics was established in 2006 by INCA, the National Cancer Institute. The standards of

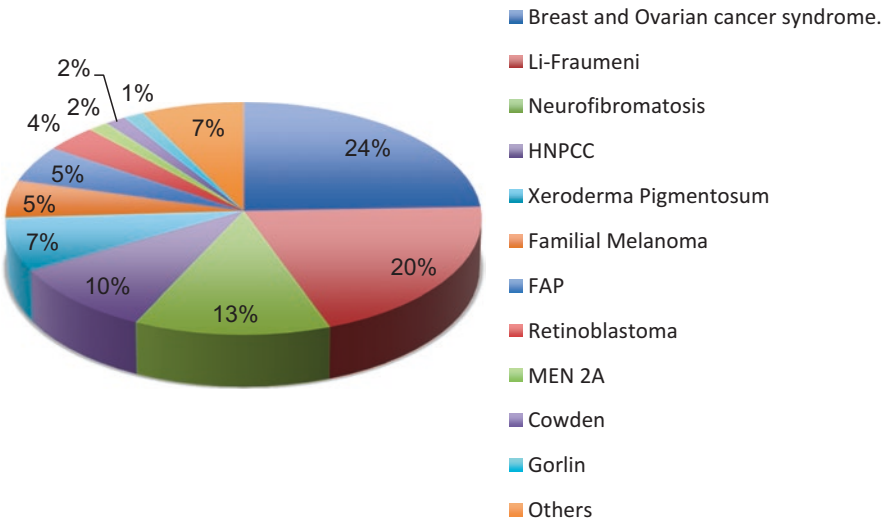

Fig. 12.5 Proportion of families diagnosed with inherited cancer predisposition syndromes at AC Camargo Cancer Center, São Paulo, 2000–2012

counseling, surveillance, testing, and care are modeled on those developed in high-resource contexts of Northern America and Western Europe, involving multidisciplinary teams of specialized medical doctors (especially geneticists and oncologists), nurses, and psychologists. These teams have developed a practice, which is adapted to the cultural and socioeconomic diversity of the populations they serve. Genetic counseling and surveillance encompass long-term follow-up of patients and families, as well as the development of specific programs for increasing awareness and early detection. Figure 12.5 summarizes the distribution of familial cancer syndromes diagnosed and followed-up over 20 years at the Department of Oncogenetics at Hospital AC Camargo, Sao Paulo. Of note, LFS represented about 20% of all diagnoses and more than 50% of familial pediatric cancer cases, due to the high prevalence of a founder *TP53* variant in the population of southeastern Brazil (see below).

In Porto Alegre, Brazil, a program of cancer genetics was established within the general university hospital. In this center, the cancer risk evaluation clinic was started within the institution's medical genetics service, the largest in the country. Over time, a multidisciplinary clinical team was developed which currently includes medical geneticists, pediatric and adult oncologists, nurses, a psychologist, and a specialist in clinical bioethics, fostering intense collaboration with healthcare professionals from many other medical specialties. In this center, as well as in the National Cancer Institute and other reference centers in the country, a specific training program in cancer genetics which was initially sponsored by the Ministry of Health and the Pan American Health Organization (PAHO) for physicians exists. Yet another significant example of development of familial cancer practice is the development of the

Oncogenetics Department of Hospital do Amor, formally named Barretos Cancer Hospital (BCH), one of the largest cancer hospitals in Brazil located in the northern part of the State of São Paulo. Since 2011, BCH has been receiving donations which enabled them to build a center capable of offering genetic counseling, preventive examinations, and genetic testing according to international standards [20].

Despite these examples of spectacular progress, it is estimated that the current provision of service in pediatric oncogenetics only covers the needs of a small fraction of the population. Indeed, the vast majority of the population relies on the public healthcare system (Sistema Único de Saúde, SUS), and public policy actions toward the identification and care of individuals and families with hereditary cancer are still insufficient [21]. To address this problem, reference centers for research and clinical care of hereditary cancer were created across the country. Currently, eight centers have been established in public hospitals in the cities of Belém (Northern region and Amazon basin), Salvador (Northeastern region), Rio de Janeiro, São Paulo, Ribeirão Preto, Barretos (Southeastern region), Brasília, Curitiba, and Porto Alegre (Southern Brazil). However, the outreach of these centers remains limited. First, they are located in urban areas and do not cover for the needs of large parts of the rural population. Second, their operation is constrained by the shortage in properly trained healthcare professionals, both medical and nonmedical. A significant limitation is that genetic counseling is only provided by medical doctors (MDs) and that the profession of genetic counselor is not yet recognized in the country. Third, SUS does not support genetic testing for hereditary cancer syndromes, and laws that have been passed to ensure that this changed were not reinforced. Public hospitals that are able to insure genetic testing may have two forms of covering the costs: (1) private donations and (2) giving out research results to the patient, which involves a greater risk of errors. An important step was made in 2012, when coverage of genetic testing by private healthcare plans became mandatory. Currently, it is estimated that 25–30% of the Brazilian population has private healthcare coverage (https://www.ans.gov.br/perfil-do-setor/dados-gerais).

12.4.2 LFS in Brazil: A Unique Founder Effect of High Population Prevalence

The high proportion of families matching broad definitions of the Li-Fraumeni syndrome (LFS) is a characteristic feature of the pattern of inherited childhood cancer in south Brazil. The genetic basis of this effect emerged in 2001, a specific *TP53* mutation at codon 377 (c.1010G > A, p.Arg337His, or R337H), was present in the germline of 35/36 children with ACT diagnosed in a large geographic area including the regions of São Paulo, Curitiba, and Porto Alegre [19]. Initially suggested to predispose only to ACT, p.Arg337His was found in the germline of patients and families matching strict or relaxed LFS criteria throughout southeastern and southern Brazil [22]. Although ACT was the leading form of pediatric cancer in p.Arg337His carriers, other childhood cancers were detected, including RMS, CPT, and other forms of CNS tumors. In a recent study on a cohort of 292 children diagnosed with tumors of

the LFS spectrum and unselected for familial history of cancer, 11 carriers of p.R337H were identified (3.7%), including 9 with ACT and 2 with CPT. Interestingly, 25.3% of these probands had a family history consistent with LFL, compared to 1.5% in sex- and age-matched, cancer-unaffected children admitted to other pediatric wards of the same institution ($p < 0.001$) [23]. In young adults, pre-menopausal breast cancer was the most frequent solid tumor in adults, followed by sarcoma. Haplotype analysis revealed the p.Arg337His variant was present on a founder haplotype in up to 0.3% of the general population in south Brazil [24–26]. It is estimated that up to 200–300,000 Brazilians may be carriers of this mutation. Several cases of homozygous carrier patients (who have inherited the mutant from both paternal and maternal side) have been reported [27]. The origin of the founder effect is not known. A plausible hypothesis is that it was introduced in southern Brazil at the time of the European colonization of this area (mid-eighteenth century) and disseminated along a major communication axis between São Paulo and Porto Alegre.

From a structural viewpoint, the p.Arg337His mutation is predicted to impair the formation of functional p53 protein oligomers in a pH-dependent manner [28]. So far, dependence upon pH or other biochemical factors has not been demonstrated in functional studies. That this variant may exert context-dependent effects is however an attractive hypothesis to explain why many carriers may not develop early cancer. Indeed, only 15–20% of the carriers develop cancer before 30 years of age (compared with 50% of carriers of "classical" *TP53* mutations), and the lifetime penetrance is estimated to 50–65% [22]. This relatively low penetrance may explain why this detrimental mutation has not been counter-selected over generations, since many carriers attain adulthood and reproductive age without developing cancer (Fig. 12.6). In addition to high-risk families, the mutation is detected in a significant

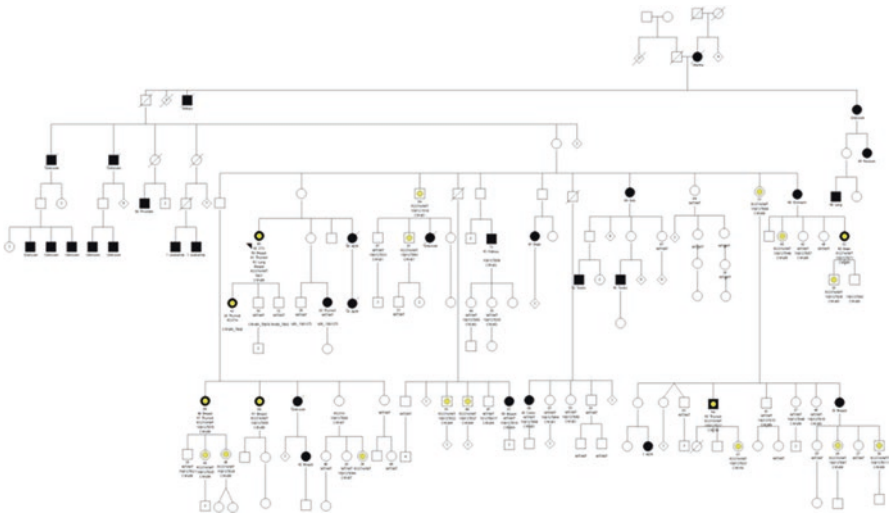

Fig. 12.6 Example of a large kindred of carriers of *TP53* p.R337H founder mutation that predispose to a variant form of Li-Fraumeni syndrome in Brazil. Confirmed carriers are highlighted in yellow. Cancer cases are shaded in black. (From [25])

proportion of cancers of the LFS spectrum occurring in an apparently sporadic manner (without evidence of family history). Analyses in Brazilian patients with apparently sporadic pediatric solid tumors or pre-menopausal breast cancer (two forms of cancers common in LFS kindreds) have shown that a significant proportion of the cases carry the p.Arg337His mutation [29].

The initial assumption that p.Arg337His was a tumor-specific mutation associated only with ACT led to the development of a newborn screening program in the State of Paraná (south Brazil) [24, 30]. Parents and relatives of positive newborns were also offered testing. The program included 171,649 newborns (of a total of about 760,000 live births in the State of Paraná during that period). The mutation was found in 461 subjects (0.27%). Participants were initially followed-up for the risk of developing ACT. Over an average follow-up of 3.5 years, 11 carriers had developed ACT, compared to 2 in the group tested as negative. Further six cases were detected in relatives of positive newborns [24]. Overall, knowledge of carrier status and awareness of ACT risk appeared to improve early detection and access to care, demonstrating a beneficial public health effect. However, another important finding of this study was that the penetrance of ACT in carriers was relatively low (2.2% by the age of 5 years). In addition, the exact penetrance of other childhood and especially adult-onset tumors in these carriers remains to be determined, posing an unsolved challenge to genetic and cancer risk counseling. Recently, an additional genetic variant in the *XAF1* gene and associated with the founder mutation in some patients was identified and may explain part of the phenotypic variability observed among carriers [31]. Because of the familial and sporadic evidence that p.Arg337His is associated with an increased risk of multiple cancers, inclusion of p.Arg337His mutation detection in state-wide newborn screening programs remains controversial when one considers uncertainties about penetrance and the current lack of evidence of the impact of risk, reducing interventions for malignancies other than ACT [32].

The public health challenge posed by the high prevalence of p.Arg337His in Brazil is daunting. It is estimated that about 4000 cases of cancer attributable to this mutation may arise in the country annually. These high figures make it difficult to address the public health impact of p.Arg337His within the classical setup of high-risk familial cancer clinics. Information and counseling are challenging because familial and individual patterns of risk are variable, ranging from no cancer (healthy carrier status) to full LFS patterns. Addressing this complex situation will necessitate the identification of reliable predictors of individual risk (genetic modifiers, metabolic biomarkers, environmental or lifestyle risk factors) and the adoption of a gradation of surveillance mechanisms commensurate with different strata of risk.

To date, there is no other example of such a large populational founder effect for a variant causing childhood cancer. It remains difficult to understand how such a major genetic effect may have occurred in an open, non-segregated population which, over the nineteenth and twentieth centuries, has welcomed wave after wave of migrants from different geographic origins.

12.5 Pediatric Cancers in Sub-Saharan Africa

Except Botswana (HDI 0.7), South Africa (0.73), and Gabon (0.7), and despite substantial recent economic growth, all countries of sub-Saharan Africa have a HDI < 0.65. This large region remains the poorest area of the world. In these countries, 43–47% of the total population is typically aged less than 15 years. Across the whole continent, the annual spending on health care per capita is below USD 100, and the vast majority comes from personal resources rather than public spending or public or private insurance systems.

There are only two nationwide population-based cancer registries with continuous operation over the past 30 years (Malawi and The Gambia). Data on the incidence of childhood cancer is scarce and poorly reliable across the subcontinent. A recent survey of 21 cancer registries has shown that, unlike high HDI countries, lymphomas, WT, Kaposi sarcoma, and RB were the most common pediatric tumors in sub-Saharan Africa. Overall, WT was the most common solid tumors, exceeding 10% of the cases of childhood cancer across sub-Saharan Africa. Non-Hodgkin Lymphoma (NHL) was the most common diagnosis in many West African countries (25–70% of the cases), whereas RB was reported as the most common childhood cancer in Congo (20.1% of the cases). Kaposi sarcoma was recorded as the most frequent cancer before age 15 years in several countries of Eastern and Southern Africa. Leukemia, sarcoma, and osteosarcoma represented between 1 and 5%. Finally, brain tumors were extremely rare (less than 1% of all diagnoses). Of note, clinical series and registries report rare early cases of adult cancers including breast, esophageal, liver, or gastric cancers before 10 years of age. In most instances, however, these cases are not histologically confirmed. This sub-Saharan childhood cancer pattern is not observed in populations of African ancestry living in more affluent areas, such as Afro-Americans in the USA (for reference, see http://seer.cancer.gov/statistics/). It is strongly influenced by the high prevalence of Burkitt lymphoma (BL) in areas endemic for Epstein-Barr virus (EBV) infection and malaria. In clinical series for sub-Saharan Africa, BL accounts for 80–90% of the cases of NHL. Another factor is the bias toward cancers, which, at late stages, produce visible external symptoms and obvious deformities, such as advanced BL, RB (with exophthalmos being a common sign at diagnosis), nephroblastoma (generally detected as large abdominal mass), and rhabdomyosarcoma or osteosarcoma (which can grow to become large lumps that disfigure or restrict functions). In contrast, pathologies such as leukemia (which requires hematological diagnosis) or CNS tumors (which require imaging) are grossly underrepresented. It would therefore be inaccurate to conclude that leukemia and brain cancers, the most common hematological and solid malignancies worldwide respectively, are uncommon in Africa. Rather, the lack of detection illustrates the dramatic lack of resources for diagnosis. The attrition caused by these cancers is most likely overshadowed by the looming high baseline of childhood mortality.

Only very few studies have rigorously documented and tested families with cancer predisposition syndrome in sub-Saharan Africa. The first description of two

confirmed African families with germline *TP53* mutations was published in South America in 2018 [33]. In both families, probands had developed ACT at an early age (10 months, 5 years), and other cancers were documented in adult relatives. The mutations identified were p.Phe109Ser, a rare variant classified as likely pathogenic, in one proband and p.Arg337His, identical to the "Brazilian founder" variant, in the other proband tested. There was however no evidence of a possible Brazilian origin for this patient.

In the next section, we briefly summarize the epidemiological and clinical findings on retinoblastoma (RB) and nephroblastoma (Wilms tumor, WT), which are the most frequently inherited childhood cancer types in sub-Saharan Africa, and we discuss current data and knowledge gaps on the role of heredity.

12.5.1 Retinoblastoma

In high HDI countries, retinoblastoma (RB) represents about 1–3% of all solid tumors before age 15 and about 10–12% of solid tumors in the first year of age. Its frequency is of about 1 per 12,500–25,000 births (1 per 16,000 in France) [34]. Incidence rates in the USA are of 3.4–4 per million person-years in the age group 0–15 years. About 60% are unilateral and nonhereditary, 15% unilateral and hereditary, and 25% bilateral and hereditary [35]. The underlying genetic defect is germline deletions or small mutations in the retinoblastoma gene (*RB1*, chromosome 13q14). Among subjects with hereditary RB, about 20% have de novo *RB1* mutations, other cases being associated with familial history of RB. Most cases are diagnosed before 1 year of age, and over 95% of the cases occur before age 5 years. The sex ratio shows a slight male predominance (1.1–1.4:1). Treatment includes chemoreduction, focal radiation therapy, and enucleation for large tumors. Survival is favorable, with >90% of treated patients alive at 5 years after diagnosis.

Estimates in different populations in the USA have suggested that rates of RB may be higher in Hispanic populations than in white populations [36]. However, it is likely that such differences may be affected by differences in diagnosis and in registration practice. Krishna et al. (2009) have conducted a comparison of age-adjusted incidence rates in 109 regions around the world from 1993 to 1997 using compiled data from the International Agency for Research on Cancer (IARC). This analysis identified a ratio of 1.12 (range: 0.35–4.15) between white populations in the USA and in Europe/Australia, 0.98 (range: 0.37–2.65) between Hispanic populations in Spain and in the USA, and 1.44 (range: 0.29–1.79) between Hispanic populations in Uruguay and in the USA. None of these differences were significant [37].

A recent international study has compiled a cohort of 4351 new patients from 278 centers in 153 countries, representing over 50% of the new cases worldwide in 2017 [38]. Most patients (n = 3685 [84.7%]) were from low- and intermediate-HDI countries. Globally, the most common indication for referral was leukocoria (n = 2638 [62.8%]), followed by strabismus (n = 429 [10.2%]) and proptosis

($n = 309$ [7.4%]). Patients from high HDI countries were diagnosed at a median age of 14.1 months, with 0.3% of them having metastasis. Patients from low HDI countries were diagnosed at a median age of 30.5 months, 18.9% having metastases. Lower national income was associated with older presentation age, higher proportion of locally advanced disease and distant metastasis, and smaller proportion of familial history of retinoblastoma. The proportion of patients receiving genetic testing was 0.9% in low-resource countries and 96.8% in high-resource countries.

There is no validated evidence of geographical or racial variations of retinoblastoma incidence [38]. Assuming a uniform rate of occurrence of pathogenic mutations in RB1, it is estimated that of the total of about 8000 annual cases of RB in the world, some 2000 occur in sub-Saharan Africa. The highest incidence rates in the world are reported for Bamako, Mali, with about 40 per 10^6 person-years. This figure should be interpreted with caution since many cases occurring nationwide and in neighboring countries may be referred to Bamako, which hosts the only pediatric oncology unit in Mali (at Hospital G. Touré) and serves as a reference center for ophthalmic diseases. A consecutive series of 55 retinoblastoma cases treated at Hospital G. Touré between 2005 and 2007 has been reported [39, 40]. The mean age at diagnosis was 50 months. The male/female ratio was 2:1, and only six patients (11%) presented with bilateral lesions. Parents had no formal school education in about 80% of the cases and did not report familial history. Of the 55 cases, 30 presented with exophthalmos, and 53 (95%) had large lesions falling in Group V of the Reese-Ellsworth classification (very unfavorable prognosis) (Fig. 12.7). Access to MRI was not available, and histological confirmation of the diagnosis was conducted in only 37 cases (67%). Patients were treated by enucleation (37 cases; 18 cases could not afford the procedure for economic or cultural reasons) and chemotherapy. In 30 months of follow-up, 31 cases (56%) were in complete remission [39].

In Kenya, a nationwide series has been reported, compiling 206 cases diagnosed between Jan 2006 and Dec 2007 in 46 health facilities across the country [41]. Using these figures, the frequency of RB in Kenya was estimated to be 1 per 17,030 live births, comparable to estimates in high-resource countries. Demographic data were available for a subgroup of 132 cases. The mean age at diagnosis was 33 months, male to female ratio was 1.5, and bilateral lesions were detected in 24% of the cases. Although familial history was collected for 98% of the cases, only 5% reported a positive history (six cases; four with bilateral and two with unilateral RB). Similar characteristics were reported for a consecutive series of 25 cases diagnosed and treated at the National Cancer Institute, Gezira, Sudan (median age at diagnosis, 33 months; bilateral lesions, 26%) [42]. Among three cases of trilateral RB (bilateral RB associated with a pineal gland lesion) reported in Burkina Faso, none acknowledged a familial history, although this form of RB is known to be systematically associated with germline mutation at the *RB1* locus [43].

These case series highlight the fact that patterns of RB in sub-Saharan Africa are dominated by poor awareness of inherited risk, late diagnosis, and late stage at presentation, compounded by lack of clinical resources and by the tendency of families to resort to traditional healers and to report to hospitals only when lesions become intractable. A qualitative survey in Kenya revealed that many survivors and parents

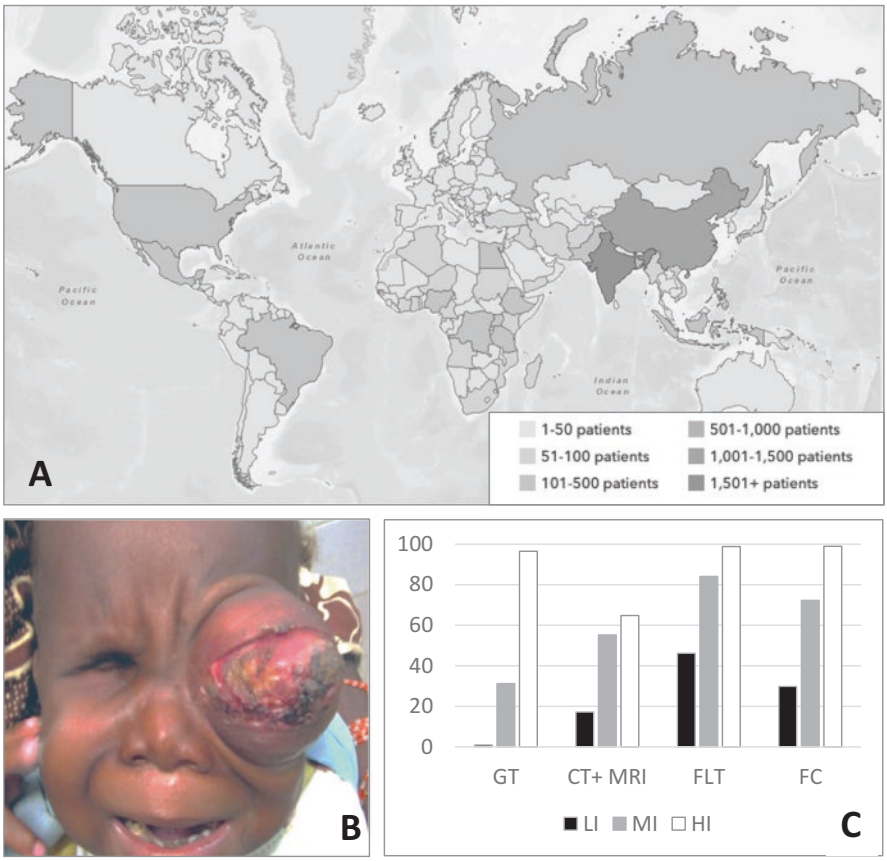

Fig. 12.7 Worldwide distribution of retinoblastoma. (**a**) Estimates of geographic distribution of retinoblastoma cases (number of cases), from [38]. (b) Presentation of retinoblastoma in a 3-year-old girl, Bamako, Mali. (From [40], reproduced with permission). (**c**) Availability of resources for RB diagnosis and treatment in low (LI)-, middle (MI)-, and high (HI)-income countries, as percentage of centers with access with these resources. *GT* genetic testing; *CT + MRI* computerized tomography+magnetic resonance imaging; *FLT* focal laser therapy; *FC* focal (intravitreal) chemotherapy

held misconceptions or had limited knowledge about retinoblastoma genetics. However, majority felt that they required more information and more simplified explanations from healthcare teams and had positive expectations toward genetic testing [44]. It should be kept in mind, however, that in these settings, few children with hereditary RB would survive and attain adulthood. Thus, the majority of RB cases in sub-Saharan Africa are likely to result from de novo mutations.

Information on mutations at the *RB1* locus in patients from sub-Saharan Africa is scarce. An analysis of the gene sequence in different human populations and in five non-human primate species has revealed a small number of haplotypes (total: 15). Of these haplotypes, ten were found in Africa, whereas a single haplotype

accounted for 63–84% of all chromosomes in non-African populations [45]. This observation suggests that the polymorphic background of the *RB1* locus may be wider in Africans than in Caucasians and Asians, raising the possibility of different genotype/phenotype correlations for *RB1* mutations in Africans. On the other hand, a recent study in Colombia has detected an increased risk of RB in relation to a maternal polymorphism in dihydrofolate reductase (DHFR) [46]. This polymorphism is associated with increased unmetabolized folate and decreased levels of red blood cell folate [47]. Folate deficiency is a prevalent condition in many parts of Africa. Further studies are needed to determine whether balancing mother's diets for folate might contribute to limit the frequency of RB in African populations.

Given that RB is a treatable disease when detected at early stages, it represents a primary target for efforts to improve pediatric cancer detection, diagnosis, genetic testing, and treatment in sub-Saharan Africa. This is the goal of a nine-point strategic program launched in 2011 in Mali, with the support of Alliance Mondiale Contre le Cancer (AMCC) and Institut Curie, Paris, France [39]. On the other hand, the lack of evidence-based recommendations for clinical management in lower-resource countries has led the International Society of Paediatric Oncology (SIOP-PODC) to set up a Global Retinoblastoma Study Group to document the presentation of treatment-naïve RB patients across the world and to generate guidelines for the clinical management of retinoblastoma in low-resource contexts [48]. Recommendations are provided for staging and treatment of unilateral and bilateral retinoblastoma and for counseling families for whom compliance is an issue. Other initiatives include "one world, one vision," a global initiative of several nongovernmental organizations to reduce childhood blindness through education and training for RB early detection and treatment [49].

12.5.2 Nephroblastoma (WT)

Wilms tumor (WT) is the most frequent renal tumor in children. About 500 new cases are detected every year in the USA. In Germany, cancer registration data for years 1993–1997 show an average number of 110 cases per year (data: Automated Childhood Cancer Information System, IARC, http://accis.iarc.fr/). Incidence rates in Europe vary between 0.5 and 2 per 10^5 person-years in the age group 0–15 years. Cancer Today estimates for the European Union indicate that it represents about 6% of all childhood neoplasms in that age group [3]. The median age at diagnosis is 3–4 years. About 10% of WT are associated with congenital abnormalities and/or overgrowth syndromes. A complex pattern known as WAGR syndrome (WT-aniridia-genitourinary anomalies-mental retardation) is found in a small proportion of children with WT. Genetic abnormalities at the *WT1* locus are detected in the majority of patients with WT (11p13). However, only a small proportion of them have a familial history of nephroblastoma, and it is unclear how many of the apparently sporadic cases may have de novo mutations or inherited mutations with low

penetrance. The frequency of germline *WT1* mutations is estimated to be 1 per 10,000 live births [35].

A case series of 42 WT cases, diagnosed over a 10-year period (1995–2004) at the University Teaching Hospital of Enugu, Nigeria, has been reported [50]. The series included 22 boys and 20 girls (sex ratio 1.1:1), with an age range of 7 months–11 years (mean: 4.1 years). All children presented with a large abdominal mass; the average duration of having this symptom before presentation was 4.7 months. Of the 42 cases, 7 were stage II, 22 stage III, and 13 stage IV. Treatment combined surgery, pre- and/or postoperative chemotherapy, and radiotherapy. Regular chemotherapy was completed for 18 cases (other eligible cases could not afford the cost of therapy). Of 25 children available for follow-up, only 10 were alive after 5 years (40%). There was no information on familial history or on associated congenital manifestations. Only 1 of the 15 cases was found to harbor a germline WT1 nonsense mutation.

In a clinical series of 72 patients with unilateral WT from Malawi, 20 (28%) had an estimated tumor diameter of more than 25 cm at presentation [51, 52]. Patients were treated with preoperative chemotherapy according to standard protocols. It was observed that this treatment caused considerable hematological toxicity and treatment-related mortality in malnourished children. Eleven of the 72 patients died during preoperative chemotherapy. Of the 61 remaining patients, 56 showed tumor mass reduction under treatment. However, 8 of these 56 tumors remained inoperable. In 2012, the French African Pediatric Oncology Group (GFAOP) published the first results of a multi-centric pilot study in eight centers in North and sub-Saharan Africa (NEPHRO-01 trial) [53]. Patients were recruited between 2001 and 2004 in a nonrandomized prospective study. Patients referred with a clinical and radiological diagnosis of unilateral nephroblastoma at stage less than stage IV were included and received preoperative chemotherapy. A selected group of 133 patients (of a total of 229 WT cases) were retained in the study. The 5-year survival was 76.7% for localized tumors and 71.6% for all study patients. These much-improved results demonstrate that there is, within the current infrastructure context, a large margin for improving WT treatment and survival in sub-Saharan Africa.

In conclusion, although WT is a common form of childhood cancer in sub-Saharan Africa, there has been so far only limited investigation on its hereditary patterns, genetic predisposition, and biological characteristics in the region. Of note, there is no evidence that aniridia, a congenital abnormality commonly associated with WT predisposition, is common in West Africa. Two studies of children reporting to eye clinics in Nigeria have reported that aniridia represented less than 1% of the diagnoses of congenital eye diseases [54]. The main obstacles for improved management of WT are the lack of awareness of signs and symptoms, the frequent resort to traditional healers delaying reporting to clinics, the advanced stage at presentation, and the incapacity of many families to afford the costs of treatment.

12.6 Conclusions and Perspectives

It can be estimated that inherited forms of childhood cancer strike between 15,000 and 20,000 children every year in the world. Less than 10% of them are correctly diagnosed and receive adequate treatment. Familial clustering is investigated in an even smaller fraction, and most families do not receive adequate information or counseling regarding cancer risk in relatives. Yet experience shows that adequate management of the risk is effective in improving early diagnosis and in reducing overall mortality. This situation is not specific to low- and middle-income countries, since lack of awareness also precludes the identification of many at-risk families in high-resource countries. The detection and management of these cancers in low- and middle-income countries has specific constraints; yet this situation offers a number of opportunities for public health actions aimed at improving awareness, training, diagnostic resources, access to care, and, ultimately, cancer survival.

There is lack of data on inherited childhood cancer in middle-income countries, despite the fact that, in the past two decades, these countries have rapidly developed high-quality medical infrastructures and have made them available for a growing proportion of their populations. The main challenge for these countries is to harness these emerging infrastructures and resources to address the burden of inherited childhood cancer. The examples of Malaysia and Brazil, described here, offer models for other countries to develop structured national and international programs. In both examples, the development of multidisciplinary networks based on, or encompassing, pediatric oncology has been essential for building a critical mass of knowledge and resources to start training dedicated medical and nonmedical staff and to initiate controlled genetic testing. The example of Brazil demonstrates that the combination of pediatric oncology, nationwide networking of oncogenetics services, and research in molecular genetics can lead to the discovery of unprecedented genetic events, such as special founder mutations with wide population impact. Two sociodemographic factors, typical of emerging countries, have influenced these developments: (1) the rapid accrual of very large populations in mega-cities of several tens of millions generates an exceptional density of high-risk subjects within relatively limited geographic areas, and (2) in the context of epidemiological transition, large kindreds with many children per generation are still very common, allowing for the detailed assessment of risk patterns in relatives of cancer patients. It is likely that the extension of pediatric oncogenetics in other large emerging countries such as India and China will uncover a whole range of new variants of known pediatric cancer predisposition syndromes as well as new founder effects associated with high risk of childhood cancer in specific population groups.

In low-resource countries where clinical resources are still scarce, the challenge of inherited childhood cancer is one component of the greater challenge of childhood cancer and children's health. The main problem in most sub-Saharan African countries is the lack of trained pediatricians. For example, in Mali, with a population of 14 million, there is only 1 pediatric oncology department for the whole country. The scarcity of human and medical resources is a major limitation for

detection and diagnosis of childhood cancer. A step forward in this respect is the development of simple tumor-specific algorithms and apps that guide the clinician through the decisional process, outlining the associations between pediatric tumors and cancer predisposition syndromes. Such simple systems, which may operate on small handheld devices such as mobile phone, could significantly boost awareness and access to information in low-resource countries.

In the absence of public or private health insurance programs, only a fraction of diagnosed patients and families can afford the costs of surgery and, whenever available, basic radiotherapy or chemotherapy. As summarized in this chapter, significant efforts have been undertaken for earlier detection and better treatment of retinoblastoma and of nephroblastoma. There is a leading role to play for international societies in supporting multinational programs aimed at training medical and nonmedical staff, structuring diagnostic practice, and implementing phase III trials for treatment modalities affordable by the target populations [48, 55].

In developing global awareness toward inherited childhood cancer, careful research must be conducted in partnership with local doctors and communities, on the perception of familial cancer risk in different cultural, ethnic, and socioeconomic contexts. Notions such as "genetic risk," as well as cultural definitions of "family" and "extended family," significantly differ among cultures and societies around the world. The simple application and translation of standards developed by decades of practice in the Western world may be inappropriate for addressing the nature and perceptions of risk in other societies. Sensitivity to these issues is particularly high when relating to children's health and welfare. The burden of these constraints has been well documented in the prevention of inherited disorders such as thalassemia [56]. Addressing these perceptions and sensitivities will require a systematic effort on how to implement high standards of bioethics and genetic counseling in different communities and societies, supported by community education to promote autonomy of individual decisions.

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Chapter 13
Frontline Ethico-Legal Issues in Childhood Cancer Genetics Research

Michael J. S. Beauvais, Karine Sénécal, Conrad V. Fernandez, Daniel Sinnett, Vasiliki Rahimzadeh, and Bartha Maria Knoppers

The 1989 United Nations Convention on the Rights of the Child states that the "best interests of the child shall be a primary consideration" in all actions concerning children and recognizes the "right of the child to the enjoyment of the highest attainable standard of health and to facilities for the treatment of illness and rehabilitation of health" [1].

Abstract Clinical research involving child participants frequently raises both legal and ethical concerns that researchers, clinicians, and parents must navigate to pursue relevant pediatric-centered investigation in health and particularly in cancer care. The foundational ethico-legal principles governing research participation provide the necessary frameworks with which to evaluate how emerging genetic technologies can serve current and future childhood cancer research. Taking the best interests of the child as the primary consideration in all decisions affecting a child, this chapter explores issues regarding consent/assent and return of results in pediatric oncology research. With a primary focus on Canada, the USA, and Europe, we

M. J. S. Beauvais · B. M. Knoppers (✉)
Centre of Genomics and Policy, McGill University, Montreal, QC, Canada
e-mail: michael.beauvais@mcgill.ca; bartha.knoppers@mcgill.ca

K. Sénécal
Independent Researcher, Montreal, QC, Canada

C. V. Fernandez
IWK Health Centre, Halifax, NS, Canada
e-mail: conrad.fernandez@iwk.nshealth.ca

D. Sinnett
CHU Sainte-Justine, Research Centre, Montreal, QC, Canada
e-mail: daniel.sinnett@umontreal.ca

V. Rahimzadeh
Stanford Center for Biomedical Ethics, Stanford University, CA, USA
e-mail: vrahim@stanford.edu

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examine the issues presented by the use of next-generation sequencing, pharmacogenomics, and biobanking and data sharing in international consortia.

Keywords Pediatrics · Ethics · Next-generation sequencing · Whole-genome sequencing · Return of results · Incidental findings · Biobanking · Pharmacogenomics

13.1 Introduction

The importance of medical research for the diagnosis and treatment of human diseases is well recognized. The involvement of human participants, however, continues to present both ethical and legal challenges for the scientific community as well as for society at large. The history of pediatric research reveals that children represent a vulnerable population that requires special protection against possible violation of individual rights and exposure to undue risks that outweigh anticipated benefit [2]. Research involving child participants has long raised a broad spectrum of concerns, which prompted their broader exclusion from research as a result [3]. Over-protectionist policies served to impede the development of pediatric-specific therapies [3] and encouraged extrapolation of data derived from adult studies [4]. Although the importance of including children in research is now well established [5], with research opportunities expanding for children and adolescents, the generation of evidence-based standards of care in pediatrics lags behind. Ethico-legal challenges are in part responsible for delays in clinical translation, such as determining appropriate protection against undue harms, the interpretation and application of the child's best interests in the research context, respect for parental authority or legally authorized representatives (LARs), as well as the developing autonomy of minors and the recognition of their growing capacity to assent, dissent, and later consent.

Advances in pediatric health research improve the way we understand child and adolescent health, childhood disease and normal development, and how they are influenced by various factors, such as the environment. Children are not small adults, and childhood cancers are seldom clinically comparable to adult cancers. Research into pediatric-specific therapies is therefore essential. For example, cancers in children include a higher proportion of hematopoietic malignancies, most commonly leukemia. Children are still developing, and the physiological immaturity of their organ systems has important treatment implications. The young age of the patients (peak incidence occurring between 2 and 5 years of age) suggests a short latency period between the first mutation and detection of tumor cells and should help to identify risk factors. By contrast, adult cancers typically result from an accumulation of mutations over a longer period of time, which are associated with environmental, occupational, and lifestyle factors, such as diet, alcohol, and smoking. Genetic analyses have further evidenced that pediatric cancers present distinctive characteristics compared to adult cancers at a molecular level [6–8], even when they look histologically similar.

Although pediatric oncology boasts a long tradition of dedicated clinical trial research dating back to the 1960s, the clinical evidence supporting standard cancer treatments for children are extrapolated from data involving adult populations [9]. Merging clinical and research aims is especially pronounced in pediatric oncology and where ethical discussions have featured prominently in the bioethics literature: "Most paediatric oncologists are investigators involved in clinical care as well as in research. As a result, a remarkable proportion of children with cancer are enrolled in a trial during treatment" [10]. As the number of child participants in cancer genetics research increases, clarifying the responsibilities of researchers, the rights of child-participants and their parents, and the consequent development of clear policies is essential in addressing emerging ethical challenges related to current technology [11].

Within this domain, three developments have direct implications for frontline ethico-legal issues in childhood cancer research. These developments include (1) next-generation sequencing (NGS) and in particular whole-genome sequencing (WGS), (2) pharmacogenomics (PGx), and (3) biobanking and data sharing in international consortia. This chapter will proceed by first giving an overview of each of these three activities. Taking the position that the best interests of the child (BIC) are the central principle in both law and ethics where children are involved, we then consider the unique challenges consent/assent and return of results present in the context of pediatric oncology research using NGS, PGx, and international biobanking approaches.

13.2 Overview of the Domains

13.2.1 Next-Generation Sequencing Technologies

WGS is a next-generation sequencing (NGS) approach that interrogates an individual's entire genome rather than just a section by way of a targeted panel. Because of its comprehensiveness, authors have described WGS as:

> A powerful research and diagnostic tool that brings with it a deluge of genetic information, including genetic data that are solicited [individual research results] and unsolicited [incidental findings], validated and non-validated, highly and poorly predictive and more or less probabilistic. One of the most urgent ethical challenges is therefore whether to disclose such genetic risk information to parents of children undergoing [WGS], particularly for conditions that do not have immediate consequences for the health of the child [12].

The increased availability of NGS has moreover drastically changed the pace of current research practice by facilitating more accurate, sophisticated, and cost-effective genetic testing. NGS has the potential to diagnose and help to identify optimal treatments for childhood cancer. It affords researchers and clinicians the opportunity to investigate molecular bases of childhood cancers, define the spectrum of long- and short-term susceptibility to resistance, minimize the side effects

of cancer therapies, and discover novel therapeutic interventions. To date, however, the promises of precision medicine remain largely unrealized [13] for diseases beyond those with known genetic predispositions [14].

NGS as part of research generates different types of results, many of which can have profound personal, clinical, familial (e.g., reproductive), or social (e.g., lifestyle) implications for the participant. Where NGS is used in oncology, its fullest potential is realized through the comparison of the tumor genome to the germline genome to isolate cancer-specific gene variants [15]. Even where the germline is not directly interrogated, germline findings may indirectly present themselves [15]. These "personal" results ultimately reveal distinct genetic patterns within one's family. Such results, therefore, can have a significant impact on both the participants and their biological relatives.

Even upon identifying genetic predispositions to familial cancer, the clinical utility of these findings may differ substantially across populations [16]. Some genes reliably predict disease risk and have well established surveillance recommendations (such as retinoblastoma). It is estimated that up to 10% of child cancer patients carry a hereditary cancer predisposition gene, which makes the discovery of clinically relevant germline variants inevitable during NGS analysis [17]. Yet, our understanding for other cancer predisposition genes is still evolving, and the evidence supporting surveillance strategies is less robust. Recent research indicates that NGS among parent-child trios has promising clinical utility in cancer care and is widely accepted when offered to families with an almost 90% acceptance rate according to one study [18].

Among the individual results and incidental findings that could be generated by NGS, there are several categories:

- Results that have analytic and clinical validity as well as clinical utility[1]:
  - For the child
  - For the members of the child's biological family
- Results indicating conditions with no known treatments but for which certain lifestyle choices or preventative measures may be taken (such as melanoma susceptibility genes and sun exposure)
- Results indicating conditions with no known treatments but which may affect reproductive choices (such as avoiding pregnancy or testing for recessive conditions with severe phenotypic abnormalities)
- Results for which the clinical significance is uncertain or unknown

[1] Analytic and conceptual validity are related but distinct concepts. The former indicates how well a test detects the presence (or absence) of a gene, while the latter is whether the test has any clinical significance for disease diagnosis, treatment, or management. For more, see Chapter 4 in National Academies of Sciences, Engineering, and Medicine, Health and Medicine Division, Board on Health Care Services, Board on the Health of Select Populations, Committee on the Evidence Base for Genetic Testing. An Evidence Framework for Genetic Testing. Washington (DC): National Academies Press (US); 2017. Available from: http://www.ncbi.nlm.nih.gov/books/NBK425808/.

- Results for which the clinical significance is of a low or intermediate probabilistic nature and thus difficult to act upon
- Results concerning familial relationships (e.g., adoption, non-paternity)

Due to the variety of "personal" (and familial) research results and incidental findings that could emerge from the use of WGS technology, its use in pediatric research is a challenge for consent and the subsequent management and communication of both research results and incidental findings.

13.2.2 Pharmacogenomics

As a specific area of research that owes its existence to developments in NGS technologies [19], pediatric pharmacogenomics (PGx) has become an important tool for assessing variability in treatment response for pediatric cancers [20]. In brief, PGx is the study of gene-gene associations in drug metabolism. The hope is that PGx will lead to better and more effective treatments for individual patients with fewer side effects. Though pharmacogenomics and pharmacogenetics are frequently used together, there are subtle differences. Generally, pharmacogenetics refers to the study of specific (and limited number of) genes related to drug metabolism and response, while pharmacogenomics refers to the general study of all of the many different genes that determine drug behavior. Both are, however, often used interchangeably, as will be the case in this chapter.

Involving children in PGx research is important primarily because children should be protected from the harms of prescribing untested drugs, or new pharmaceutical products. They require special consideration because they differ psychologically and physiologically from adults, and their sensitivity to drugs may vary considerably depending on their age of development. Despite the fact that children's reactions to drug trials cannot be easily extrapolated from adult trials, "up to 80% of all medications currently prescribed in Canadian paediatric hospital settings are administered 'off-label', meaning that use deviates from the dose, route of administration, patient age, and/or indication" [9, 21].

Pediatric oncology exemplifies the integration of PGx analysis in the understanding of variability and toxicity in response to chemotherapy. For example, clinicians now routinely evaluate at the time of diagnosis whether or not the child with acute lymphoblastic leukemia possesses a variant of thiopurine methyltransferase (TPMT). Children with TPMT are poor metabolizers of the drug 6-mercaptopurine. With TPMT testing, oncologists can modify doses accordingly and so prevent known adverse reactions. The attention to interpatient variability using PGx tests allows for appropriate dosing in order to prospectively prevent bone marrow suppression and maximize uninterrupted delivery of chemotherapy. In turn, disease control and long-term outcomes are expected to improve [22, 23].

Cisplatin is yet another frequently cited example of the impact of PGx in pediatric oncology. Cisplatin is an anticancer agent used to treat childhood cancers

involving the central nervous system, among others, including brain tumors, neuroblastoma, and germ cell tumors. Cisplatin is frequently associated with severe side effects including nephrotoxicity, neurotoxicity, and permanent ototoxicity. Until recently, clinicians could predict population-level risk for cisplatin side effects but could not do so for an individual child. In addition to predictable effects of high-dose cisplatin on hearing [24], there is some evidence of ototoxicity following standard doses of cisplatin in children with polymorphisms in several coding enzymes that metabolize this chemotherapeutic agent [25–27].

PGx is still a nascent field despite promising advancements in identifying novel polymorphisms associated with altered drug metabolism and toxicity. Clinical translation of PGx testing requires robust evidence and guidance for addressing the myriad psychosocial factors relevant to returning PGx results. Rigorous attention should be paid to providing protections to children against unwanted side effects while preserving overall curative benefits. Researchers have also called for greater international collaboration in PGx research. Taken together, increasing statistical power and improving subgroup analyses to capture heterogeneity among study populations will lead to better evidence regarding efficacy and safety [28]. It is important that researchers accurately convey the limits of PGx research to study participants and their families so as to avoid any therapeutic misconception of its clinical applicability. We finally note that the application of NGS to PGx research also raises issues concerning return of results. Specifically, there are two categories of results: (1) results for which the clinical significance is uncertain or unknown and (2) results for which the clinical significance is of a low or intermediate probabilistic nature and thus difficult to act upon.

13.2.3 Biobanking

For childhood rare diseases such as cancer, sharing sample and data among scientists is a crucial way to ensure that studies have sufficient statistical power to generate generalizable knowledge [29, 30]. Research infrastructures, such as biobanks, are key to realizing this goal. Although biobanking is a common and well-established resource for research [31, 32], it takes on new ethical dimensions when the data is intended for future research. Such research often involves investigations with no direct connection to the participants, even in the international context. The ethical issues related to the use of stored tissue samples for future research are amply discussed in the literature [33, 34] and primarily involve delineating their governance, determining the scope and duration of consent, ensuring privacy protections, providing feedback, and commercializing findings. The International Cancer Genome Consortium (ICGC-ARGO) is one such international example. One of the Consortium's objectives is to increase the availability and accessibility of data, across all cancers, for the entire research community [35]. Both consortium members and other international researchers have access to ICGC-ARGO data through both open and controlled access databases. The Pan-Cancer Analysis of Whole

Genomes (PCAWG) analyzed data from ICGC-ARGO and The Cancer Genome Atlas (TCGA) through cloud computing infrastructure, which enabled researchers to study 2,658 whole-cancer genomes in a path-breaking research project [36]. Such large-scale research collaborations highlight the need for harmonized data sharing rules for researchers across jurisdictions [37].

Research using biobanked samples and data from pediatric patients poses additional ethical issues to those raised by research involving adults. Children may have limited capacity, if any, to understand the short- and long-term consequences of sharing their data. Biobank researchers are thus challenged to assess that adequate assent was provided at the time of data or specimen contribution. They are later re-challenged to pursue informed consent from adults to the continued use of data collected when they were children [32]. It seems, however, that most adolescents have sufficient capacity to consent to biobank participation [38, 39]. It remains unclear, however, whether oncology patients differ in their capacity to participate in data sharing decisions in the context of biobanking research. Researchers and regulators alike increasingly face challenges in addressing the broader familial implications of genetic results derived from child research participants. Whether incidental to the primary aims of the research or a direct target, these results can create tensions between researchers' obligations to respect the child's future autonomy interests, including their right to data privacy, and any potential benefits of disclosing clinically significant results to parents, siblings, and other biological relatives. Mediating between these concerns is rendered more difficult when one considers that pediatric disease studies using biobanked samples may not only be geographically distant but also have limited relevance to the aims of the original study for which consent for the return of results was obtained.

13.3 Best Interests of the Child

As stated at the outset of the chapter, the best interests of the child (BIC) is the "the central ethos for decisions concerning children participating in genomic research" for both ethics and law [40].The *Convention on the Rights of the Child* (*CRC*) is the most important source of the BIC in law. The *CRC* is the most successful United Nations treaty, with 196 States Parties, all of which having also ratified the Convention, except for the USA and South Sudan [41]. The *CRC* embodies the idea that children themselves are rights-bearers for whom special protections are warranted owing to their vulnerability [42]. Parents and other authorized individuals who act in the child's stead are to have the BIC as their basic concern [1].

The *CRC* presents a broad spectrum of rights for the child. It is both protective and emancipatory [43]. The BIC is frequently understood as a protective concept, especially in the biomedical context where it is broadly accepted that pediatric research must carry only minimal risk to the child participant [5, 29, 44, 45]. "Minimal risk" is a relative concept that is determined by reference to the intervention's nature, scale, its expected impact on the health of the participant, the current

state of biomedical science, and the availability of alternative procedures, as well as importantly in relation to the current status of the participant's condition [46]. Children may participate in clinical trials with higher-than-minimal risk where there is a prospect of the child, or the group the child represents, benefitting from the research [4].

On the other hand, principles, such as the right for a child to be involved in decision-making in a manner that is appropriate for their age and capacities ("right to be heard"), highlight that children may exercise their burgeoning autonomy. Indeed, the only limit on the child's right to be heard is their capacity for expression [47]. While the protective and emancipatory concepts may appear to be contradictory at first glance, the United Nations emphasizes their compatibility [48]. That is, appropriately including a child in decisions affecting them serves to further the child's best interests. Thus, it is ideal to respect children's autonomy while safeguarding their best interests until both "collapse altogether and the child's wishes…become the controlling factor" [49].

The protective and emancipatory aspects of BIC are furthermore compatible to the bioethical principle of "respect for persons." The latter extends the individualist concept of autonomy to include protections for those with diminished or lack of autonomy. Thus, respect for persons includes the "dual moral obligations to respect autonomy and to protect those with developing, impaired or diminished autonomy" [50].

The primacy of the BIC standard can be heightened for children with cancer. Developmental milestones toward evolving one's decision-making capacity may be delayed and, in exceptional cases, never reached. Children with a life-threatening diagnosis, for example, face an even shorter timeframe within which to exercise their rights and participate meaningfully in decision-making. On the other hand, experience by virtue of the diagnosis and treatment of cancer may provide the child with insights not normally present in a child of their age. This experience may enhance their ability to participate meaningfully in subsequent decision-making as regards treatment.

The two principal foci of this chapter—consent/assent and return of results—are heavily influenced by considerations for the child's welfare and best interests. The consent and assent process must embrace the panoply of interests that intersect when a child participates in research. Surrogate decision-making provided by parents or other lawfully authorized representative (LAR) is an ethically and legally sanctioned practice insofar as parents and LARs act according to the child's best interests [51]. Consent, however, is a necessary but insufficient condition for the participation of children in research [45, 50]. The assent process, by which the child signals their agreement to participate, activates their right to be heard as protected by the *CRC*. Similarly, a child's dissent to clinical and research decisions should also be respected in line with the BIC.

The relationship between ethical and legal norms in Canada with regard to biomedical research is complex, and law is frequently silent on many issues, with ethical norms then filling the lacunae [52]. As regards consent/assent in the research context, the legal norms in most Canadian provinces are silent on the issue of the

participation of minors [51]. Thus, the two-pronged consent/assent process is often the standard relied upon until the child reaches the age of legal majority. The province of Quebec is a key exception, where minors 14 years or older may consent on their own to research participation if it involves minimal risk (conforming to the international principles cited above) and their circumstances justify participation [53]. There is risk, however, that the age of majority standard unjustly impinges on the right to autonomous decision-making among adolescents and mature minors who are fully capable of understanding the consequences of research participation. Researchers should be aware of their local obligations and the requisite measures to explore assent and consent if their research involves minors.

13.4 Consent and Assent

The preceding section discussed that research involving minors generally requires consent of the child participant's parent or LAR as well as assent of the incapable child participant where appropriate. Where applicable, this dual requirement is owed to the fact that research is not necessary for the child's care, and so children cannot consent on their own. Ethical and legal norms view the parent or LAR as the guardian of the child's best interests in the research process. The dual requirement differs significantly from the clinical context, where children who are able to understand a proposed procedure and its consequences, viz., capable or mature minors, are able to consent on their own.

Children's limited ability to understand the consequences of oncology research participation, coupled with the uncertain informational risks associated with NGS technologies, gives rise to unique consent- and assent-related considerations. The following section will discuss these issues in depth. The former will detail consent-related ethico-legal issues viewed through the lens of NGS, PGx, and biobanking, including the potential consent from competent minors. The latter will discuss the same applications but with a focus on assent.

13.4.1 Consent

The need to obtain informed consent from research participants is no different in research related to childhood cancer genetics. The decision of parents or legal guardians to consent on behalf of their children must be taken in the child's best interests. As a general rule, parents are legally considered the best-placed persons to make such decisions, and parental choices should be generally respected [54]. The informed consent process and its associated information-giving duties ensure that parents and LARs are able to indeed act in the child's best interests.

Although research with children, including research that makes use of NGS, enjoys wide acceptance, PGx and international biobanking present some specific

issues related to consent. Participation in biobanking that uses NGS and in PGx research exposes children to the risks of receiving genetic information unrelated to the current study, which are included in the data collection algorithms. PGx studies furthermore present unique challenges to the consent process. This is largely because trial sponsors routinely encourage optional sampling and sequencing during clinical trials for an "add-on" study [55] and may retain data and/or samples for future research purposes [56]. These "add-on" studies for future analysis of samples are often conducted simultaneously with clinical trials. There are two relevant types of PGx studies for our purposes: (1) research into specific genes and polymorphisms that are thought to be associated with drug response and (2) exploratory research where the scope has yet to be defined but requires long-term retention of the participants' samples [57].

13.4.2 Governing the Consent Process

The risks associated with participation in PGx studies are primarily informational rather than physical [22, 58]. This distinguishes PGx from standard clinical trials and renders them similar to biobanking in certain regards. Moreover, the sharing of biological materials and their possible irretrievable "banking" outside of the country still raises substantial controversy in the context of pediatric research [59].

The informational risks for pediatric genetic research include potential insurance discrimination and loss of information privacy or security [21]. Both Canada and the USA have federal laws that prohibit genetic discrimination in employment and insurance contexts. Researchers should be prepared to inform their participants of these privacy and discrimination protections and risks as part of the informed consent procedures [124]. Specific to PGx and beyond the information-giving obligations during the consent process, researchers and clinicians should understand not only the potential harms and benefits but also the complex terminology surrounding genetics and PGx. This is largely due to the fact that the task of implementing PGx research is often left to clinicians, which remains a major challenge [58, 60].

The management of "add-on" studies and sample storage are increasingly important features of the consent process involving PGx. In cases where a single consent form is used, the terms of use for any biological material for genetic analysis should be explicitly defined. The consent document(s) must discuss the potential to withdraw from the add-on PGx study with no adverse impact on a participants' access to clinical care or their participation in the larger clinical trial. Where a single consent form is used for both the clinical trial and the PGx "add-on" study, the sections describing each type of participation must be clearly outlined in the consent form with respective signatures agreeing to both [61]. To better highlight the difference between the primary clinical trial and the add-on PGx study and thus reduce the potential for confusion among participants, some would recommend that researchers use a separate consent form for the clinical trial and for the PGx add-on study [62].

13.4.3 Consent Specificity

As with any research project, the aims, data coding strategy, and security measures should be specified when obtaining consent from participants [62]. Where it is unclear, as in frequently the case with biobanks, how stored samples and data will be used in future research, the scope of consent may be problematized. In Canada, broad consent has emerged as an accepted, and indeed essential, practice for biobanking, including where children participate [32]. Considering the purpose of informed consent is to ensure comprehension and voluntariness of participating in the research project at hand, it is recommended that the potential secondary use of that is intended for ongoing exploration after the initial study be clearly stated in the consent form [61]. In addition, the informed consent form should outline the possibility that samples could be used for future research (and commercialization), and the potential scope of the specific activities, including international data sharing, should be described in as much detail as possible at the time the consent is sought [57]. The consent should also describe any protections that are in place for future use, including oversight by data access committees and research ethics boards. It is important to clearly describe the implications of broad consent, and ideally the participant should be given the choice of participating only in the current research or in future research as well, unless the policy and aims of the research dictate otherwise.

While the next section is dedicated to a discussion of the return of results, it is important to note that return of results has consequences for the consent process. International norms state that a research project's return of results policy should be outlined to potential participants during the consent process [63]. Competent adolescents or their parents should be informed about whether and how the results will be disclosed, their general right not to know their results, and the exceptions described above in the best interests of the child. Parents should be informed that clinicians (perhaps in consultation with the ethics committees) might be in the position to override parents' refusal to receive clinically significant research results. This exercise of authority on the part of physicians poses additional logistical challenges. The timing of the return of results (e.g., during research, or at its completion) and the process (e.g., by whom the results will be communicated, to whom, with which services (e.g., consultation with genetic counselor), should also be discussed [64].

13.4.4 Governance

As a research infrastructure, governance of biobanks' collections of samples and data is a central concern not only for researchers but for participants themselves [65, 66]. While biobanks' material transfer agreements usually foresee sample destruction or the return of any remaining material, there still remains some risk of future misuse. Some authors argue that the potential harm posed by data sharing can be

mitigated through limiting the data to be shared, removing identifiers from datasets, and maintaining ongoing oversight and governance [67]. Other possible proposed solutions are "to regulate access to genetic data from all types of biobanks, develop robust data security measures, and criminalize the misuse of genetic information, as has been done in the Swedish law on genetic integrity" [68–71]. Yet, this initial Swedish law on biobanking has since been replaced due to the restrictive conditions it imposed [72]. Criminal prohibitions nevertheless remain in this domain. Since 2018, for example, the UK has criminalized the re-identification of de-identified personal data [73]. While the reliance on criminal law sends a strong message that informational risks are taken seriously, it is no substitute for ongoing governance and security measures.

For example, the Canadian Health Measures Survey (CHMS) [74] includes children under 14 years of age (starting at age 3) and compiles longitudinal statistics on the prevalence of childhood asthma, cancer, chronic disease, and other disabilities. As part of the CHMS, biological samples (e.g., blood or urine), physical measures, environmental factors (e.g., quality of household air), and questionnaires (e.g., regarding sociodemographics, nutrition, etc.) are collected. This data is meant to be representative of the pediatric population "whether they are healthy or not, and provide a better picture of the actual health of Canadians." The study methods for CHMS ensure ongoing updates and that only data stripped of identifiers is released for research and stores biological samples "for further analysis of measures at a later date."

Beyond protecting participants, governance implicates the information-giving duties of researchers. For all of the criticism of broad consent [75], biobanks actually have the potential to be more attuned to participant autonomy than short-term clinical trials with specific consent due to their ongoing communication platforms. Many established biobanks publicize information regarding which researchers access the bank and for what research and provide general research results on their websites. This is the case, for example, of the UK Biobank (United Kingdom) [76] and of CARTaGENE (Quebec) [77]. The possibility for recontact, together with revealing aggregate research results on their websites, both serve to highlight research developments and remind them of their rights, including the right to withdraw.

13.4.5 Reconsent

Issues of reconsent arise because the child participant is unable to consent on their own to research. While the parent or LAR provides consent, what about where stored samples or data are used in research after the child reaches the age of majority? It is interesting to note that the CHMS no longer attempts to directly recontact minors for reconsent at majority due to logistical and privacy issues. Instead, the CHMS sends a notification to participants upon reaching the age of majority and allows them to opt-out of the study [38]. One advantage of longitudinal biobanks is

that consent to recontact the parents, and later the mature minor, is an option discussed during the recruitment and the initial consent process.

There is little data on how many participants in pediatric oncology research generally would welcome recontact following initial consent, nor what percent would agree, initially via broad consent to future unspecified research with ethics review and ongoing governance. A recent systematic review of the literature found that recontact to continued data use at the age of majority was the single most mentioned practice of responsible data sharing [78]. Yet there still remains a diversity of approaches in the literature. One commentator supported broad parental consent on the condition that children provide their own consent at the age of majority [79]. In contrast, it has also been argued that the agreement of the child's parents or LARs at the time of obtaining tissue samples is sufficient to justify the continued storage and use of the samples after the minor has reached the age of majority [80]. Still others have argued that waivers should be granted more often in the case of stored pediatric tissue samples [81].

There is empirical support for these positions as well. One study reported that 67% of adults whose samples had been stored during their minority would not be concerned about the use of their sample/data after they reached adulthood [82]. In a study examining the opinions of adolescents, 89% thought that children who were not involved in the initial enrolment decision should be recontacted once they reach the age of majority [83]. Finally, a study examining the opinions of adolescent patients in oncology, cardiology, and orthopedic clinics found that 50% of adolescent patients and 64% of their parents believe that reconsenting pediatric patients once they reach the age of majority is "'Important' or 'Very important'" [84]. Irrespective, "fundamental research may eventually also result in knowledge advances that may allow medical progress without this being foreseen. It may be permissible to assume that such research is part of the original parental consent, although some disease categories may be more sensitive than others, such as psychiatric diseases" [85].

The diversity of approaches suggests that issues relating to reconsent and recontact are context-specific. It nevertheless remains essential that researchers be clear at the time of enrolment about what will happen when the child participant reaches the age of majority.

13.4.6 Assent

As already stated, and in addition to parental consent to research, the assent of minors should be obtained when feasible [44, 45, 50, 86]. This is supported by the American College of Medical Genetics and Genomics (ACMG) statement on engaging older pediatric patients in the genome sequencing consent process, which states that adolescents should be involved in this process in the clinical and research settings [87]. It is clear that the mental capacity to make informed decisions that have implications later in life does not instantaneously spring, fully formed, into

existence; rather, this competency develops over time. Respecting the child's developing autonomy in an ethically robust fashion is achieved through engaging them in discussions on the proposed purposes of the research that are tailored to their level of understanding [88]. Yet, given the complexity of the consequences associated with research participation, in particular the potential informational harms, coupled with the remarkable complexity of genetic information itself, is it possible to seek meaningful assent in the context of pediatric cancer research in genetics and genomics? As children's capacity to absorb and rationalize information matures, so should the level of information provided to them, and so incur a greater emphasis on assent. It should be remembered that parental consent and the assent of a child represent cumulative criteria such that a lack of either will disallow participation in research [51].

We note that the emotional maturity and comprehension to assent for the return of research results is unlikely to emerge until mid-adolescence to late adolescence. For a younger child, the amount of information disclosed need not be extensive, and in-depth discussion of the otherwise controversial issues in genetic testing would in all likelihood not be meaningful for young children [89].

As biobanking does not offer any immediate health benefits and must therefore pose no more than a minor increase over minimum risk to child participants [90], is it permissible from the point of view of parents to engage children in such infrastructure science at all? Longitudinal population studies in particular allow for the study of child development, and much of the empirical evidence collected to date would seem to suggest "yes." One pediatric oncology biobank reporting on parental views found that 86% of parents would agree to send tissue anywhere in the world but preferred the research derived thereof to have pediatric aims [69]. Moreover, 98% would consent to sharing samples for genetic research if it improved their child's health, and 76% would consent even if there was no direct health benefit for their child. Another study reported that 54% of parents of pediatric cancer patients would renew consent if their child's stored tissues were repurposed for research [91].

13.5 Return of Results

The return of results is a central and ongoing issue when applying NGS technologies. In any type of research that uses NGS, the information generated may go well beyond initial research questions. This "additional" information may include incidental findings that have implications for the child and/or his or her family [58, 92]. We note that there is no consensus regarding how and when minors should be made aware of results. Therefore, it is left to the parents to determine when it is appropriate to inform their children about results in accordance with their age and/or level of maturity. The development of clear policies for return of results is thus essential.

Ethico-legal considerations for return of results center largely on the complexity of consent and assent and respect for future autonomy more broadly. When research involves young children, results should be returned to their parents or LARs. When

the research project involves older adolescents, results should also be returned to them (with parental approval) in a manner appropriate to their level of development, comprehension, and maturity. Where incidental findings involve particularly sensitive information (e.g., non-paternity) or familial "actionable" implications, disclosure should be determined on a case-by-case basis. Researchers conducting longitudinal studies could offer to return results once participants have reached a level of maturity that enables them to adequately comprehend and fully appreciate implications of the information provided. This section will examine the considerations and different positions in Canada, the USA, and Europe regarding return of results. As with previous sections, NGS is considered broadly with specific reference to international biobanking and PGx research.

As briefly discussed in the previous section, parents are responsible for consenting to the return of individual research results or incidental findings when a minor child participates in genomic research. Most parents of children and adolescents with cancer believe that they have a right to the research results—including study-related issues directly relevant to their participation—and even more so as the child reaches the age of majority [93]. Indeed, parents may frequently have a legal right to access research data when such data have clinical relevance [108]. Evidence also suggests that most adolescents would like access to incidental genomic findings, often endorsing "the justification that knowing this information would enable them to plan for their futures" [94]. In this way, parents and adolescents have a twofold rationale for supporting the return of all research results. Both parties receiving results may experience direct benefits impacting immediate quality of life, allowing clinicians to initiate preventative measures, closely monitor disease progress, and inform future healthcare decisions. A second rationale is more indirect and includes augmenting public understanding of the significance of participating in research and reducing secrecy surrounding the research enterprise itself [93, 95].

13.5.1 Positions on Secondary Findings in the Clinical Context in Canada, the USA, and Europe

Where NGS technologies are used on children, there is an ethical tension between a child's autonomy, a child's health, parental choice, and a healthcare professional's duties [96]. For pediatric cancers, the germline may be interrogated, and so a large amount of data, even those that are clinically pertinent, concern only the future health of that child (e.g., information on carrier status, eventual development of late-onset diseases, likelihood of developing common diseases), not his or her current health.

The use of NGS in childhood cancer research, as in other contexts, "creates a different calculus than that which was envisioned with predictive testing for a familial condition" [97]. There is considerable debate concerning a researcher's duty to report secondary findings that are valid and clinically actionable or whether

responsible data analysis should hold researchers to a duty to hunt for clinically significant results [98]. Other commentators adopt the position that parents should have the option to decide whether they want to receive genetic results that reveal a child's predisposition to incurable adult-onset disease [99, 86, 100]. This is contested by professional pediatric societies.

In 2013, the ACMG endorsed an obligation for certified laboratories to actively search and report findings for specific mutations in a predesignated list of genes, including when such findings concerned children or adolescents [97]. In essence, this argument underscores the professional obligation to avoid harms when they are present. A physician, for example, would be obliged to disclose incidental findings of all the conditions on the ACMG list. It should be noted that the ACMG list represents a minimum "floor" of return—many institutions return more than the recommended variants (59 at the time of writing) insofar as tests have clinical and analytic validity and based on professional judgment. Having been the source of much consternation [101], the ACMG revised their position in 2015 to allow opting out of such analysis and return of "incidental findings" [102]. Thus, while at first children like adults were subject to the "no opt-out" of the gene panel, the 2015 guidelines allow parents to opt-out on behalf of their child. The 2016 update (ACMG SF v2.0) has maintained this position, in contrast to positions in Canada and Europe [103, 104, 85].

Whereas the ACMG policy creates a bioinformatics pipeline that generates secondary findings, Canadian and European guidelines suggested creating a bioinformatics pipeline that minimizes the potential for secondary findings [105, 106]. The Canadian guidelines underscore the distinction between childhood-actionable and adult-actionable findings. They recommend that laboratories searching for secondary findings should return results for conditions that are highly penetrant *and* medically actionable in childhood. The Canadian College of Medical Geneticists (CCMG) recommends that a child's risk for adult-onset genetic conditions should not be communicated to parents unless they request disclosure *and* the disclosure could prevent serious harm to the health of a parent or family member [105].

European guidelines provide less precise norms but nevertheless emphasize the child's right to an open future [106]. The European Society of Human Genetics' (ESHG) clinical WGS policy states: "[i]n case of testing minors, guidelines need to be established as to what unsolicited information should be disclosed in order to balance the autonomy and interests of the child and the parental rights and needs (not) to receive information that may be in the interest of their (future) family" [106].

We note that there is insufficient data on the presupposed harms of the disclosure of conditions to either children or family members. (We further discuss psychosocial harms below.) We do not support the ACMG's categorical position on two grounds. It currently precludes the possibility of parental opt-out, giving precedence to parental authority above a professionally determined BIC standard. The ACMG position also limits return to a relatively short list of conditions that is strictly curated. Further, the mandatory panel, imposed as a condition for lab analysis of WGS, constitutes a form of obligatory secondary screening that may be contrary to the BIC. Healthcare professionals, along with parents and children, must work

together to guard the child's best interests on a case-by-case basis—not through categorical positions.

13.5.2 Return of Incidental Findings in Research

Generally, incidental research findings are to be communicated only if (1) the results are scientifically valid, that is, they possess analytical and clinical validity, (2) the results have significant implications for the health of the minor, and (3) effective prevention or treatment is available and should be initiated during childhood or adolescence to prevent harm. When these criteria are met, the return of research results more appropriately aligns with the BIC. As such, parents should not refuse the return of results if these conditions are met [64]. Parents' refusal to receive results that fit this set of criteria could be considered as grounds for considering medical neglect insofar as such refusal may preclude the possibility of treating actionable, childhood-onset disorders.

Human research guidance and professional organizations agree that parents not be given the option to opt-out of receiving important, clinically validated health information that is medically actionable during childhood. The 2018 Canadian *Tri-Council Policy Statement* (*TCPS-2*) states, for example, "authorized third parties, who, by law, must always exercise their authority in the best interest of the child, must receive any findings for the child that are actionable immediately or during childhood" [50].We thus interpret the BIC as determined by professionals to take precedence over a parent's authority to consent.

Researchers should communicate incidentally discovered information regarding the susceptibility for adverse responses to the treating physician, and it would be for them to decide whether to inform the family/patient. Such findings may be particularly salient for future health outcomes of pediatric cancer patients yet are only "actionable" upon drug prescription. The psychosocial impact of returning a PGx result may not rise to the same existential significance as that of disclosing one's risk for an adult-onset disorder. It also does not invariably foreclose on the child's right to an open future, supporting PGx disclosure in most cases. PGx data has moreover shown itself to be highly effective in supporting clinical decision-making with pediatric patients [107]. If relevant and clinically validated, information generated during PGx research then presents a strong case for inclusion in the health records of child participants.

Nevertheless, the arguments for returning research results in all circumstances run counter to most existing clinical ethics guidelines. It is counterintuitive to establish a policy allowing parents to receive more information in a research setting than in a clinical context. The legal landscape also echoes this position—many Canadian provinces do not give individuals a legal right to access research data about themselves but do so with regard to clinical information [108, 109]. In addition, research results, by nature, may be neither patient-specific nor significant in delivery of care because they are often not clinically validated. Research purports to produce

generalizable knowledge, and, consequently, the return of individual results and incidental findings should be considered only under certain specified conditions. In the case of longitudinal biobanks, which usually support observational or epidemiological research that involves little or no contact with the researcher, it is "harder to validate the findings clinically and thus they may be exempted from this rule [to return results]" [85].

The use of NGS moreover generates a large amount of raw data that research participants may request. The issue of parents requesting their children's raw sequence data has raised concerns regarding the utility and potential uses of such data [108, 110]. It has been recommended that in such circumstances, individuals be given general information on the implications, risks, and limitations of raw genomic data, that they are not clinically validated, and that research projects should consider a standardized process for handling such requests. This process might include mentioning the availability of raw data—distinguished from the return of secondary findings—on the consent form [110].

Recall the return of results brings to the fore issues of pediatric autonomy and may challenge parents' rights to access their child's results [111]. There are also operational concerns with return. It has been observed that "masking or tailoring the reporting of such information according to the age of the patient could place an unrealistic burden upon laboratories facing increasing volumes of clinical sequencing" [97].

Our position of "no return unless clinically actionable during childhood" subscribes to two further considerations: familial needs and the future psychosocial needs of the child.

13.5.3 Familial Considerations

The incidental information generated by NGS raises disclosure considerations for biological family members of participants. Since NGS reveals so much about both parties simultaneously, are biological family members justified in having access to the child's results? For example, if a child tests positive for the BRCA1 or BRCA2 mutation—and the tests are clinically validated—should this finding be disclosed to the parent who is likely to have this mutation as well?

Although disclosure will not immediately impact the child's health per se, it can inform subsequent testing and surveillance of the parent (or in sibling), which many have argued is in the best interests of the child. This, in fact, occurred during The BabySeq Project. A BRCA2 mutation was identified in a sequenced infant and where the original research protocol only included the possibility of returning childhood-onset conditions [112]. The research team obtained IRB approval to recontact the parents to offer the return of the adult-onset findings. The research protocol was then modified to mandate the return of results of both childhood- and adult-onset disorders.

As the BabySeq example demonstrates, communicating these results can have significant consequences for biological relatives for whom the success of preventive health measures (cascade screening, regular follow-up, early intervention, etc.) depends on returning results. This potential situation therefore complicates professional duties to disclose in that (1) the risks of communicating findings may have significant psychosocial consequences for the future health of the child and (2) preventing harm to other biological individuals involves timely return of results. Of course, there may also be significant benefit to the child should the information obtained through return of genetic results lead to life-saving, preventative action that avoids severe illness or death of their parent [100].

Thus, benefits to the child and improving parental or familial health and life outcomes may justify the psychosocial risk of communications to biological family members and perhaps at the expense of the child's loss of future autonomy. The Clinical Sequencing Exploratory Research (CSER) Consortium Pediatrics Working Group's 2019 statement agrees that a child's potential loss of an "open future" must be weighed against other pertinent factors when making predictive genetic testing decisions and should not be regarded as absolute [113]. Similarly, the 2018 *TCPS-2* outlines that in extraordinary circumstances, disclosure to relatives may be warranted and participant disclosure preferences "may be subject to overriding considerations… (e.g., if genetic research reveals information about a serious or life-threatening condition that can be prevented or treated through intervention)" [50]. Consequently, a "one-size-fits-all" approach as followed in BabySeq may not be appropriate. Indeed, BabySeq's approach has been the subject to vigorous critique for its incompatibility with the emerging international consensus that children not be tested for adult-onset conditions [112]. General principles, such as the BIC, respect for persons, beneficence, etc., should be applied with due regard to the circumstances of each case.

Yet, it could be argued that if genetic information has relevance for future reproductive choices of the parents, there should be a way to inform them [114]. The findings with significance for relatives has the potential to change fundamental aspects of the legal duty of confidentiality as well. Scholars have proposed a composite duty that balances confidentiality with a duty to care for genetic relatives [115], which has recently been followed for the first time in England and Wales [116]. While the legal debate has been constrained to the clinical context thus far, reworking legal duties of confidentiality may eventually come to affect the research context. For the time being, it seems appropriate not to expect a higher degree of disclosure in the research context than is the established norm in clinical practice, which in most cases means disclosure is limited to the proband themselves.

13.5.4 Psychosocial Consequences

The disclosure of information about the future health of a child when there is no treatment or preventive measure could engender more harm than benefit [117]. The potential psychosocial harms stemming for disclosure of unactionable findings is a

central concern for the CCMG position for children as discussed above [105]. Disclosing such results could create anxiety for the parents while occasioning psychological distress for the child. Results may also have important consequences beyond immediate physical health and might include stigmatization, impaired self-esteem, and anxiety [50, 96, 97].

If unactionable information causes parents to "see" their child differently, then the BIC are likely not furthered in the disclosure of such information. In this vein, psychosocial research has begun to confirm that the "therapeutic gap" between the availability of genetic information and the comprehensibility of such information leaves families in disquieting situations [118]. In the context of prenatal screening, it has been observed that parents' distress increases upon learning that there is no information available to them to understand a finding of variants of unknown significance [119]. While psychosocial research must go further in probing these issues, it is likely similar responses will be observed with parents outside the prenatal context.

There has been further concern that such knowledge would adversely affect parent-child interactions [120]. Parents are also inherently conflicted in their decision-making for multiple psychosocial reasons, including that they "share genes" with their child. Any results might directly impact their own health (e.g., the discovery of a BRCA1 mutation) or as concerns reproductive decisions (e.g., the discovery of a dominant condition). The central role parents assume in the child's life means that this evolving field of empirical research on the most appropriate methods of return merits special consideration in the bioethics community.

We, along with others [12], support the view that the child's "right" to an open future can be preserved through designing prudent disclosure policies. Accordingly, we posit that the pediatric guidelines for genetic testing in the clinical setting are likewise needed in the research context, along with an emphasis on establishing restrictions to delimit the return of certain research results in the latter. We also propose that researchers should be obligated to share results that meet a validity threshold akin that adopted in a clinically certified laboratory. In contrast to clinical settings, we do not believe that researchers have an obligation to actively search for these clinically significant mutations but do have an obligation to pursue them if encountered in the course of research. We also underscore the need to work with primary care physicians, clinicians from various subspecialties, genetic counselors, trainees, residents, ethics review boards, as well as the media and patient groups to enhance genetic literacy and interpretability of the results.

13.6 Conclusion

Childhood cancer has an important impact on health, economic, and social welfare systems as a consequence of its associated mortality and morbidity. Early diagnosis and prompt access to appropriate treatment are vital for patient survival and are more effective uses of healthcare resources than tertiary interventions.

Similar to all new health technologies, NGS, biobanks, and PGx present challenges and opportunities. The oncogenomics research arena represents a unique opportunity to study the fundamental biology of childhood cancers and leverage precision approaches to treatment and care. With its widespread integration in pediatric cancer research, oncogenomics provides the potential to identify molecular bases of childhood cancers, define the spectrum of the long- and short-term susceptibility to resistance, minimize adverse side effects of treatment, and elucidate novel therapeutic interventions.

Our position that the best interests of the child remain at the forefront of both research and clinical considerations is echoed in international conventions (*CRC*) and national position statements (e.g., US Presidential Commission for the Study of Bioethical Issues) [121]. The protection of children through consent and assent, along with disclosure of medically actionable results as they relate to NGS, biobanking, and PGx, must be carefully considered if these approaches are to become the mainstay in future pediatric genetic research. Ongoing empirical "contextual" and child-specific research is needed to inform best practices that effectively meet these challenges.

The fused clinical research context childhood cancer research epitomizes the learning health system [122]. The quest for generalizable knowledge may also lead to personalized and significant insights about individuals that may guide their clinical care. As NGS technologies increase in availability within health systems and "genomic medicine" becomes a reality, the need for rethinking ethico-legal principles increases. Indeed, scholars who benefit from genomic medicine in a public health system have an ethical duty to share information so that informational resources increase in availability and utility for others [123]. We are all invited to reconsider the roles and correlative rights and duties of all actors in the collective research and health system to ensure the global distribution of scientific benefits in genomics is equity-enhancing.

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Chapter 14
Genetic Counseling and Testing

Jaclyn Schienda, Carly Grant, Sharon E. Plon, and Katherine A. Schneider

Abstract Genetic testing options are now widely available to pediatric cancer patients and their families. Thus, it is important for clinicians to become familiar with the features and patterns of hereditary cancers, the elements of the genetic testing process, and the implications of the various genetic test results. In this chapter, we discuss the indications for cancer genetics referrals, motivations for genetic counseling, collection of family history information, cancer risk assessment, pretest counseling, possible genetic test results, posttest counseling, and special genetic counseling situations. We conclude with two case narratives that help illustrate the complex nature of pediatric cancer genetic counseling and testing.

Keywords Pediatric cancer genetic counseling · Pediatric cancer syndromes · Pediatric cancer risk assessment · Pediatric cancer genetic testing · Pediatric cancer genetic test results · Risks and benefits of genetic testing · Psychological impact of genetic testing · Li-Fraumeni syndrome · Hereditary retinoblastoma · Familial adenomatous polyposis · Hereditary paraganglioma and pheochromocytoma · *DICER1* syndrome

J. Schienda (✉)· K. A. Schneider
Pediatric Cancer Genetic Risk Program, Center for Cancer Genetics and Prevention, Dana-Farber Cancer Institute, Boston, MA, USA
e-mail: Jaclyn_Schienda@dfci.harvard.edu

C. Grant
Center for Cancer Risk Assessment, Massachusetts General Hospital, Boston, MA, USA

S. E. Plon
Departments of Pediatrics/Hematology-Oncology and Molecular and Human Genetics, Baylor College of Medicine and Cancer Genetics Clinic, Texas Children's Hospital, Houston, TX, USA

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14.1 Introduction

Genetic counseling and testing have been incorporated into many pediatric oncology programs with patients and families obtaining genetic risk assessments, undergoing testing, and requiring follow-up for positive results. Thus, it is important for clinicians to become familiar with the features and patterns of hereditary cancers, the elements of the genetic testing process, and the implications of the various genetic test results.

Genetic counseling is the art and science of providing cancer risk information to families in a manner that is both informative and empowering [1]. This is not an easy task given the nature of the topics being discussed. Discussions of genetic risk can invoke a number of reactions and concerns for the parents of a child with cancer. However, most families appreciate the opportunity to learn their child's genetic risk status and recognize that these results have potential implications and benefits for the entire family [2].

In this chapter, we discuss the indications for genetics referrals, motivations for genetic counseling, collection of family history information, cancer risk assessment, pretest counseling, possible genetic test results, posttest counseling, and special genetic counseling situations. We end with two case narratives that help illustrate the complex nature of pediatric cancer genetic counseling and testing.

14.2 Indications for Genetics Referrals

Genetic testing is currently available for a number of hereditary cancer syndromes which confer increased risks for specific childhood cancers (Table 14.1).

It is estimated that at least 8–10% of pediatric cancers are associated with a hereditary cancer predisposition syndrome [5–11]. However, this may be an underestimate, as studies have focused analysis on previously described cancer predisposition genes and also identified many variants of uncertain significance (VUS) that may later be reclassified as pathogenic. In addition, a study of childhood cancer survivors found that 29% (109 of 370) were eligible for genetics referral or follow-up based on family history of cancer, tumor type, medical history, or family history of another condition [12]. It is important to note that pediatric cohort studies have shown that tumor type and family history are not always reliable in predicting who will test positive for a pathogenic (P) or likely pathogenic (LP) variant or in what gene [6, 7]. Therefore, many institutions refer every child diagnosed with cancer or a brain tumor for cancer-specific genetic counseling.

If institutions do not have the ability to see every child with a cancer or brain tumor, the presence of any of the following features in the child's personal or family history increases the likelihood of a cancer predisposition syndrome and should prompt a genetics referral (Table 14.2).

Table 14.1 Examples of hereditary cancer syndromes that predispose to childhood cancer [3, 4]

Syndrome	Gene (s)	Inheritance	Main features
Beckwith Wiedemann syndrome (BWS)	11p15.5 imprinting defects, *CDKN1C*	AD	WT, HB, NB, RMS, hemihypertrophy, macroglossia, macrosomia, omphalocele, ear lobe creases/pits
Bloom syndrome	*BLM*	AR	Growth deficiency, skin lesions, immunodeficiency, LK, LYM, cancer
Constitutional mismatch repair deficiency (CMMRD)	*MLH1, MSH2, MSH6, PMS2*	AR	CO, CNS, LK, LYM, CAL
Cowden syndrome (*PTEN* hamartoma tumor syndrome)	*PTEN*	AD	ENDO, THY, BR, CO, KID, trichilemmomas, papillomatous papules, macrocephaly, ASD
DICER1 syndrome	*DICER1*	AD	PBB, OV sex cord-stromal, genitourinary RMS, THY, cystic nephromas
Familial adenomatous polyposis (FAP)	*APC*	AD	CO, PTC, HB, MB, adenomatous polyposis, desmoids
Fanconi anemia	*FANCA,C,D1,D2,E,F,G,I,J,L, M,N,O,P,Q,T,U,V,W; FANCR; FANCB*	AR; AD; XL	BMF, physical abnormalities, LK, SCC
Hereditary leiomyomatosis and renal cell cancer (HLRCC)	*FH*	AD	Leiomyomas of the skin and uterus, RCC
Hereditary neuroblastoma	*ALK, PHOX2B*	AD	NB
Hereditary paraganglioma/ pheochromocytoma syndrome (PGL-PCC)	*SDHA, SDHB, SDHC, SDHD, SDHAF2, MAX*	AD	PGL, PCC, GIST
Hereditary retinoblastoma	*RB1*	AD	RB,SARC, MEL
Juvenile polyposis syndrome (JPS)	*BMPR1A, SMAD4*	AD	Juvenile hamartomatous GI polyps, CO, STO, SB
Li-Fraumeni syndrome (LFS)	*TP53*	AD	SARC, CNS, ACC, BR, CO, LK, many other cancers
Multiple endocrine neoplasia, type 1 (MEN1)	*MEN1*	AD	PTH, PIT, GEP, carcinoids, lipomas, facial angiofibromas

(continued)

Table 14.1 (continued)

Syndrome	Gene (s)	Inheritance	Main features
Multiple endocrine neoplasia, type 2 (MEN2)	*RET*	AD	MTC, hyperparathyroid, PCC
Neurofibromatosis Type 1 (NF1)	*NF1*	AD	OG, neurofibromas, CAL, MPNST, PCC, JMML
Neurofibromatosis type 2 (NF2)	*NF2*	AD	Vestibular schwannomas, CNS
Nevoid Basal Cell Carcinoma syndrome (Gorlin)	*PTCH1*	AD	BCC, jaw keratocysts, palmar/plantar pits, lamellar calcification of falx, MB
Nijmegen Breakage syndrome	*NBS1*	AR	microcephaly, growth deficiency, characteristic facial features, ID, LYM
Peutz-Jeghers syndrome (PJS)	*STK11*	AD	PJS hamartomatous GI polyps, CO, STO, BR, PAN, LG, SCT, lip/buccal mucosa/fingertip freckling
Rhabdoid tumor predisposition syndrome (RTPS)	*SMARCB1/SMARCA4*	AD	Rhabdoid tumors, schwannomas, CNS
Rothmund-Thomson syndrome	*ANAPC1/RECQL4*	AR	Rash, sparse hair, small size, juvenile cataracts, OS, BCC, SCC, MEL
von Hippel-Lindau syndrome (VHL)	*VHL*	AD	Hemangioblastoma of retina and CNS, renal and pancreatic cysts, PCC, RCC
WT1- related Wilms tumor syndrome	*WT1*	AD	Wilms tumor, GU anomalies +/− ID (WAGR, Denys-Drash), aniridia (WAGR)

AD autosomal dominant; *AR* autosomal recessive; *XL* X-linked

ACC adrenocortical carcinoma; *ASD* autism spectrum disorder; *BCC* basal cell carcinoma; *BMF* bone marrow failure; *BR* breast cancer; *CAL* café-au-lait spots; *CNS* central nervous system; *CO* colon cancer; *ENDO* endometrial; *GEP* gastroenteropancreatic tract tumors; *GI* gastrointestinal; *GIST* gastrointestinal stromal tumor; *GU* genitourinary; *HB* hepatoblastoma; *ID* intellectual disability; *JMML* juvenile myelomonocytic leukemia; *KID* kidney; *LG* lung cancer; *LK* leukemia; *LYM* lymphoma; *MB* medulloblastoma; *MEL* melanoma; *MPNST* malignant peripheral nerve sheath tumor; *MTC* medullary thyroid cancer; *NB* neuroblastoma; *OG* optic glioma; *OS* osteosarcoma; *OV* ovarian; *PAN* pancreatic cancer; *PCC* pheochromocytoma; *PGL* paraganglioma; *PIT* pituitary tumors; *PPB* pleuropulmonary blastoma; *PTC* papillary thyroid cancer; *PTH* parathyroid tumors; *RB* retinoblastoma; *RCC* renal cell carcinoma; *RMS* rhabdomyosarcoma; *SARC* sarcoma; *SB* small bowel cancer; *SCC* squamous cell carcinoma; *SCT* sex cord tumors; *STO* gastric cancer; *THY* thyroid; *WAGR* Wilms tumor-aniridia-genitourinary anomalies-retardation; *WT* Wilms tumor

Table 14.2 Personal and family history features which increase risk for cancer predisposition [12–14]

Tumor is rare or uncommon
Bilateral tumors or two separate primary cancers
Age at diagnosis is younger than typical
Cancer type suggestive of a hereditary cancer syndrome
Patient has noncancerous features associated with a hereditary cancer syndrome
Patient has additional unusual physical features or developmental problems
Tumor testing revealed a possible germline pathogenic/likely pathogenic variant
Family history of similar or related cancers with any of the features listed above
There is a known pathogenic/likely pathogenic variant in the family

The Patient's Tumor Is Rare or Uncommon Children with rare tumors have an increased likelihood of carrying a germline P/LP gene variant [5, 15, 16, 14, 17] (Table 14.3). Examples include medullary thyroid carcinomas (*RET*) and rhabdoid tumors (*SMARCB1*). Certain benign tumors also increase the likelihood of a hereditary gene P/LP variant, such as paragangliomas and gastrointestinal stromal tumors (*SDHB*, *SDHC*, and *SDHD*). The presence of a rare tumor type should routinely generate a cancer genetics referral even if there is no additional history of cancer in the family.

The Patient Has Bilateral Tumors or Two Separate Primary Tumors Children who develop bilateral tumors have an increased likelihood of having a hereditary cancer syndrome. As an example, a child with bilateral retinoblastoma has a presumed 100% likelihood of having hereditary retinoblastoma [19]. Also, children who develop two separate primary cancers (not related to treatment) have an increased risk for having a hereditary cancer syndrome. For example, a child who developed an acute lymphoblastic leukemia (ALL) at age 4 and an osteosarcoma at age 12 has an increased likelihood of having a *TP53* P/LP variant [20].

The Patient's Age at Diagnosis Is Younger than Typical Hereditary cancers tend to occur at younger ages than sporadic cancers. For example, a child diagnosed with unilateral retinoblastoma has a higher likelihood of carrying an *RB1* P/LP variant if diagnosed at 3 months rather than 3 years of age [19]. In addition, the risk of a hereditary cancer syndrome is increased for children who have developed malignancies which are typically adult-onset cancers. For example, children or adolescents who have developed colorectal polyps may have one of these rare genetic

Table 14.3 Examples of rare or uncommon tumors with associated hereditary cancer syndromes [5, 18, 17, 16, 14] (Table 14.3)

Type of tumor	Hereditary cancer syndrome(s)
Adrenocortical carcinoma	Li-Fraumeni syndrome
Choroid plexus carcinoma	Li-Fraumeni syndrome
Colon cancer or polyps	Peutz-Jeghers syndrome, juvenile polyposis syndrome, familial adenomatous polyposis (FAP), constitutional mismatch repair deficiency syndrome (CMMRD)
Gastrointestinal stromal tumor	Paraganglioma-pheochromocytoma syndrome, neurofibromatosis I
Hepatoblastoma	FAP, Beckwith-Wiedemann syndrome
Medulloblastoma	FAP, nevoid basal cell carcinoma syndrome, CMMRD, rhabdoid tumor predisposition syndrome, Fanconi anemia
Neuroblastoma	Hereditary neuroblastoma, Beckwith-Wiedemann syndrome
Neurofibroma	Neurofibromatosis I, neurofibromatosis II
Ovarian sex cord-stromal tumor	*DICER1* syndrome, Peutz-Jeghers syndrome
Paraganglioma	Paraganglioma-pheochromocytoma syndrome
Pheochromocytoma	Paraganglioma-pheochromocytoma syndrome, von Hippel Lindau, multiple endocrine neoplasia II, neurofibromatosis I
Retinoblastoma	Hereditary retinoblastoma
Rhabdoid tumor	Rhabdoid tumor predisposition syndrome
Sarcoma, bone	Li-Fraumeni syndrome, Rothmund-Thomson syndrome, hereditary retinoblastoma
Sarcoma, soft tissue	Li-Fraumeni syndrome
Thyroid cancer, medullary	Multiple endocrine neoplasia II
Wilms tumor	Familial Wilms tumor, Beckwith-Wiedemann syndrome, *DICER1* syndrome

conditions: familial adenomatous polyposis (FAP), juvenile polyposis syndrome (JPS), Peutz-Jeghers syndrome (PJS), or constitutional mismatch repair deficiency (CMMRD) syndrome [21–23].

The Patient's Cancer Type Is Suggestive of a Hereditary Cancer Syndrome Certain forms of cancer are recognized features of specific cancer syndromes and thus may warrant referrals for genetic counseling and testing. Examples include hypodiploid acute lymphocytic leukemia (ALL) (associated with *TP53* P/LP variants) [20], sex cord-stromal ovarian tumors (associated with *DICER1* P/LP variants) [24], and clear cell meningioma (associated with *SMARCE1* P/LP variants) [25].

The Patient Has Noncancerous Features Associated with a Hereditary Cancer Syndrome Certain benign findings/features may be suggestive of a specific

hereditary cancer syndrome. Examples include lip freckling (associated with PJS) [22], congenital hypertrophy of the retinal pigment epithelium (CHRPE) (associated with FAP) [22], and congenital thumb abnormalities (associated with Fanconi anemia) [26].

The Patient Has Additional Unusual Physical Features or Developmental Problems Children with additional dysmorphic features or developmental delays may have a hereditary cancer syndrome or possibly a chromosomal deletion syndrome. For example, a small number of children with hereditary retinoblastoma have 13q deletion syndrome, which causes both an increased risk for cancer and also developmental issues [19]. Another example is *PTEN* hamartoma syndrome, which can be associated with macrocephaly, autism, and severe developmental delay [27]. Dysmorphic features can be variable and quite subtle and may require a careful evaluation by a pediatric geneticist.

Tumor Genetic Testing Has Revealed a Possible Germline Pathogenic/Likely Pathogenic Variant Tumor karyotyping and DNA analyses typically reveal multiple P/LP variants and variants of uncertain significance (VUS). The majority of these variants are confined to the tumor (i.e., somatic) rather than being present in all of the person's cells (i.e., germline). However, these tumor analyses can also identify P/LP variants that are in the germline and are linked with a hereditary cancer syndrome. Therefore, further genetic testing may be warranted to determine if certain variants found in the tumor are of somatic or germline origin [28].

Immunohistochemistry (IHC) for the presence or absence of specific proteins is also routinely performed on tumors to help characterize the specific tumor type and also provide risk information for hereditary cancer syndromes. For example, IHC for the mismatch repair proteins MLH1, MSH2, MSH6, and PMS2 is frequently performed on colorectal cancers. An abnormal result with loss of one or more of the proteins can be suggestive of Lynch syndrome and may warrant germline genetic testing for the Lynch syndrome genes.

There Is a Known Pathogenic or Likely Pathogenic Variant in the Family Children, who have not had cancer but are members of a family known to carry a specific gene P/LP variant, may have up to 50% chance of carrying the familial variant. These children should also be referred for a cancer genetics consultation to discuss and arrange targeted genetic testing.

The Patient Has a Family History of Cancer Children with cancer who also have a family history of cancer, especially when diagnosed before age 50, should be routinely referred to cancer genetics. Children who, themselves, have not had cancer but who have concerning family histories of cancer can also be referred for a genetics consultation; however, in this situation, other relatives who have had cancer may be better candidates for genetic testing.

14.3 Family Motivations for Genetic Visits

There are many different reasons why families seek genetic counseling in the pediatric cancer setting. The family's motivations may overlap with those of their providers; however, there may also be differences. Parents may be motivated to seek genetic counseling and testing to answer the following questions:

Why Did This Cancer Occur? Identifying an underlying genetic condition as the cause of cancer can help parents cope with their child's cancer diagnosis by decreasing uncertainty and providing some control over the situation. However, parents may not be looking for a literal answer to this question; rather, they are searching for the meaning of their child's diagnosis [29]. Negative results, although not yielding any definitive answers, may still be reassuring to families. Parents may also be reminded that often there is no conclusive answer (or single reason) for why the child's cancer occurred.

Will the Genetic Results Impact Treatment Decisions? In certain situations, genetic test results can help inform treatment or surgical decisions. For example, in Li-Fraumeni syndrome (LFS), exposure to radiation appears to increase the risk for a second tumor [20, 30]. Therefore, oncologists tend to avoid or minimize radiation treatments whenever possible for patients with LFS. In addition, personalized chemotherapy options may become available by knowing the germline status of certain gene variants. Although germline genetic testing for treatment decision-making is not currently standard of care in pediatric oncology, it is likely to become an important consideration in the future.

Is the Child at Increased Risk for Additional Tumors? Most hereditary cancer syndromes increase the risks for more than one type of cancer. Recognizing these additional cancer risks would allow the child to have access to early detection and risk reduction strategies. For instance, a child who carries an *SDHB* P/LP variant is at increased risk for developing paragangliomas, pheochromocytomas, gastrointestinal stromal tumors, kidney cancer, and thyroid cancer [31, 32]. Recommended monitoring would include imaging and biochemical studies to screen for these tumors. For several of the hereditary cancer syndromes, screening guidelines do exist; however, most of these guidelines are based on expert opinion rather than empirical data [15, 33, 34]. Since medical guidelines for many hereditary cancer syndromes are evolving rapidly, centers often recommend annual follow-up visits.

Do Other Relatives Have Increased Risks for Cancer? Identifying a hereditary cancer syndrome also provides risk information for the patient's siblings, parents, and other extended family members. If a P/LP variant is identified, other family members can be tested to clarify if they, too, have the increased cancer risks associated with the gene variant. In addition, the proband's future offspring will be at increased risk (often 50%) for inheriting the variant. Some individuals may wish to use the information to make family planning decisions. This can include the use of

reproductive technologies such as prenatal testing or pre-implantation genetic diagnosis.

Does My Child Have the Genetic Condition Present in Our Family? If a child is at high risk to have a cancer gene P/LP variant previously identified in the family, targeted gene testing can be performed. The genetic test results will help clarify that child's cancer risks and whether he/she requires any additional or specialized cancer monitoring. For example, a parent who has FAP and a known *APC* P/LP variant may request genetic counseling and testing to determine if any of his children inherited the familial *APC* variant. One clear motivation for parents who want their children tested for the genetic condition in their family is the hope that their children will not have it.

14.4 Collection of Family History Information

The multi-generational family history of cancer is the foundation of the genetic counseling and testing discussions. The pedigree is a standardized diagram of the family relationships and relevant medical information for the proband (patient) and relatives (Fig. 14.1). Pedigrees typically include the following information: name, age, gender, cancer status, type of cancer and age at diagnosis and, if deceased, the age and cause of death. Additional information provided in the pedigree can include genetic testing results, cancer monitoring results, and possible environmental or medical risk factors.

There are many benefits to depicting the family history in a pedigree format, which include the ability to assess, at a glance, the pattern of cancer in the family, to update and revise information easily, and to use the standardized nomenclature for clinical and research purposes. Genetic counselors also recognize the other benefits of the pedigree discussions, such as gaining rapport with the patient and parents, learning the "family stories" about the cancer experiences, and gaining insight into the family's attitudes, fears, and knowledge about their cancer risks [35, 36].

For the purposes of cancer risk assessment, cancer genetic counselors will gather the following family history information:

Details About the Patient's Cancer Diagnosis and Other Features Genetic counselors will start the information gathering process by learning specific details about the child's cancer diagnosis. This information should be confirmed with medical record documentation. This line of questioning will also help reveal if there are any unusual features of the tumor or the age of onset. Genetic counselors may also ask birth history and a review of systems, focusing on whether the child has any skin lesions, dysmorphic features, or other significant issues or problems, which may suggest an underlying genetic syndrome.

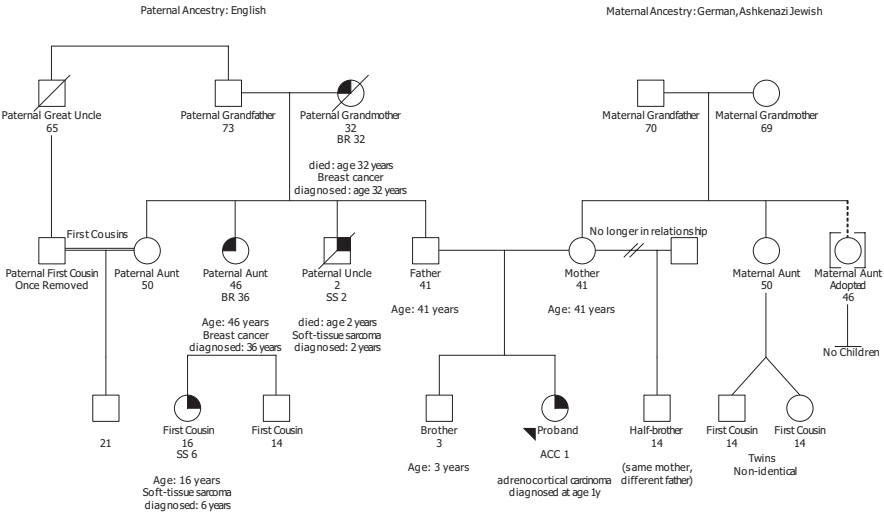

Fig. 14.1 Sample pedigree: Women (*circles*), men (*squares*), proband (*arrow*), individuals with cancer (*partially shaded*), relationships (*connecting lines*), deceased (*single diagonal line through gender icons*)

Information About Affected Relatives Genetic counselors will collect information about the other cases of cancer in the family, including the relationship of each relative to the proband, the ages at diagnosis, and the specific type of cancers which were diagnosed. Genetic counselors may work with the child's parents to help obtain documentation of the key cancer diagnoses in the family.

Information About Unaffected Relatives Genetic counselors will also gather information about relatives who have not had cancer, including whether they have had any other significant medical problems, benign lesions, or prophylactic surgeries. If, for example, the family reports that three relatives were found to have colonic polyps, it would be important to determine the number, size, and type of polyps as this could be suggestive of a polyposis syndrome.

14.5 Cancer Risk Assessment

In pediatric cancer genetic counseling, the main purpose of collecting the family history information is to determine the likelihood of an underlying hereditary cancer syndrome and to assess the utility of genetic testing or other medical screening tests.

Families will be classified as having a high, moderate, or low risk of having a hereditary cancer syndrome. Traditionally, families assessed at high or moderate risk are appropriate candidates for genetic testing. However, personal and family history does not always predict who will test positive for a hereditary cancer syndrome, and thus, there may be value to offering genetic testing to all children with cancer, especially as the cost of genetic testing continues to decrease. The high-, moderate-, and low-risk categories are described below:

High Risk Based on the child's personal and/or family history of cancer, a child who meets (or almost meets) the clinical criteria for a specific hereditary cancer syndrome has a high likelihood of carrying an associated gene P/LP variant. Figure 14.2 denotes a pedigree that is illustrative of a high-risk family. Screening recommendations for the child, and possibly other relatives, would often follow published guidelines for the syndrome, regardless of the genetic test results. Resources for providers include a series of expert reviews from the American Association for Cancer Research Childhood Cancer Predisposition Workshop published in *Clinical Cancer Research* in 2017 [34], the National Comprehensive Cancer Network (NCCN) [33], GeneReviews [37], Online Mendelian Inheritance in Man (OMIM) [4], and UpToDate [38]. For example, a child with five or more juvenile gastrointestinal polyps will be followed with guidelines for juvenile polyposis syndrome, even if no specific gene P/LP variant is identified [21].

Moderate Risk In moderate-risk families, the child's personal or family history raises concerns about a possible hereditary cancer syndrome but does not meet clinical criteria for the syndrome. The best way to clarify the cancer risk in these families is often to perform genetic testing. If the genetic test results are positive, then the screening would follow published screening guidelines for the specific syndrome. If the genetic test results are negative, then the screening would be based on the pattern of cancer in the family. Figure 14.3 is an example of a moderate-risk pedigree. In many centers, *APC* testing is offered to all children diagnosed with hepatoblastoma, even if there is no family history of colorectal polyposis.

Low Risk It is estimated that up to 90% of cancers are *not* due to an underlying germline P/LP variant, although with improved recognition of family histories and emerging technologies to identify cancer susceptibility genes, more genetic causal associations are being recognized. In general, families assessed to be at low risk for having a hereditary cancer syndrome do not need to consider genetic testing nor do they need any additional cancer screening based on the family history. However, as discussed earlier, an argument can be made for offering genetic testing to all children with cancer with appropriate genetic counseling. In Fig. 14.4, a child with acute lymphocytic leukemia at age 5 whose only family history of cancer is a grandmother who developed lung cancer at age 60 would be reassured as to the low likelihood that the child has an underlying genetic predisposition to cancer.

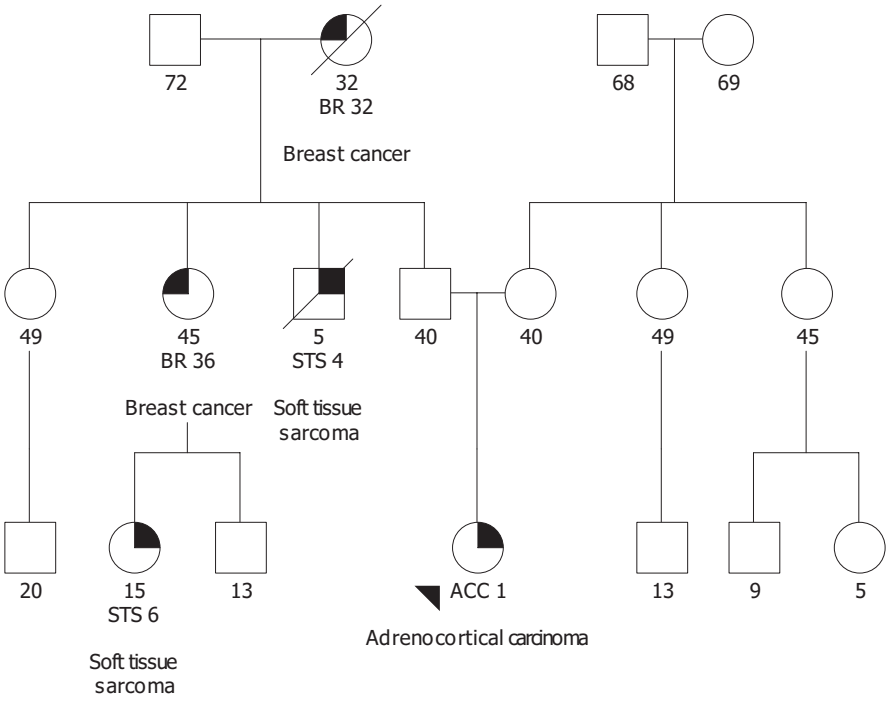

Fig. 14.2 High-risk pedigree. This proband has a personal and family history which is highly suggestive of Li-Fraumeni syndrome. This child has an estimated 80% chance of having a *TP53* P/LP variant and should be offered testing

14.6 Pretest Counseling Discussions

The main goal of the pretest discussion is to ensure informed consent for the genetic test. If the child has the developmental capability of understanding the information and testing, he/she is asked to provide assent for genetic testing [39–41] with the amount and level of detail tailored to his/her needs [42]. Some parents prefer that the initial discussions about testing be conducted without the child present, especially if the child is young or tends to be anxious.

Uptake of genetic testing varies depending on provider recommendations, parental attitudes toward testing, perceived benefits of testing (such as the availability of established screening or risk-reducing measures), and the child's current health status. The severity and risks associated with the syndrome in question may also affect uptake. For example, most parents of children diagnosed with retinoblastoma are interested in having their children undergo *RB1* testing. In comparison, when surveillance options for LFS-related tumors were limited, only 55% of at-risk individuals in one study chose to have *TP53* testing [43].

The following topics are typically discussed at a pretest counseling session:

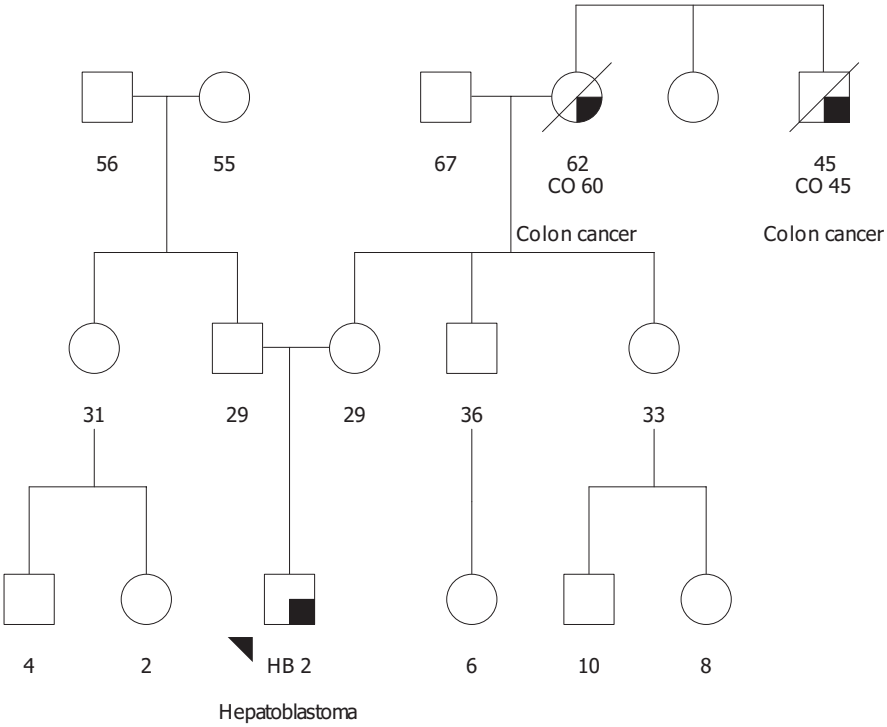

Fig. 14.3 Moderate-risk pedigree. This proband has an increased chance of having FAP based on his personal and family history and should be offered *APC* testing. However, the older ages of the colorectal cancer diagnoses and the lack of cancer or polyps in the mother make it less likely that this proband has FAP

Description of the Syndrome and Test If there is one or more specific syndrome(s) of concern, the genetic counselor will describe the genetic condition(s) in detail, including the associated cancers and lifetime risks of these cancers, current screening and prevention strategies, and the potential implications for family members (such as siblings, parents, future children, and extended family members) also carrying a P/LP variant if one is identified in the child. Genetic counselors will also provide information about the genetic test and the possible test results (see next section). This discussion may also include a brief primer to help patients understand the basic concepts of inheritance and carcinogenesis. As broad, multi-gene panel testing becomes more common than single gene testing, less information about specific syndromes is given, but general concepts about cancer risk and management are emphasized [44].

Testing Logistics The counselor will explain the genetic testing process. This includes the type of specimen needed (usually blood or saliva), the cost of the test and whether it is covered by insurance, how long it will take for results to be available (usually 2–4 weeks), and how the results will be disclosed (by telephone,

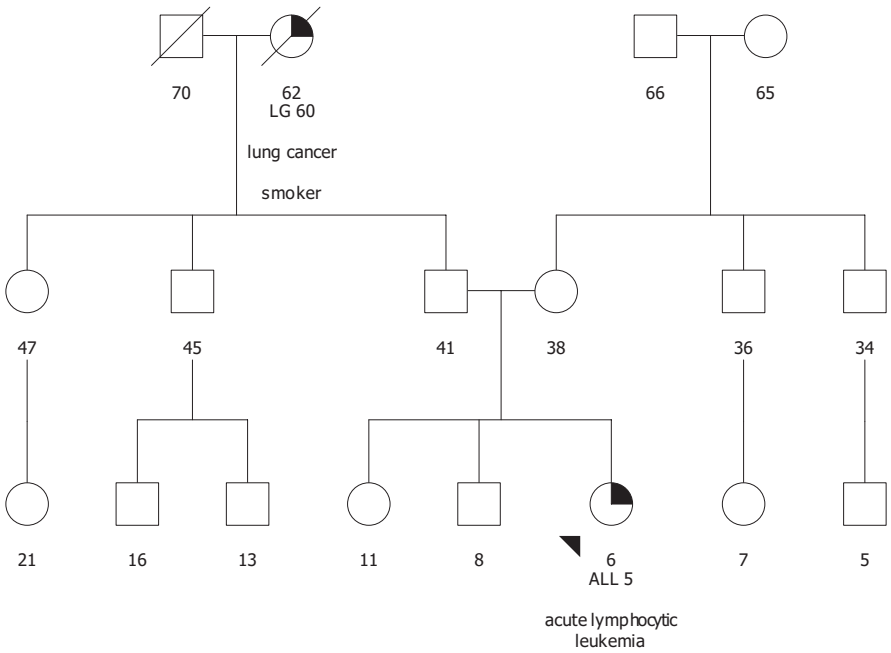

Fig. 14.4 Low-risk pedigree. This proband has less than 1% risk of having a hereditary cancer syndrome based on her personal and family history of cancer

in-person, etc.). The common types of genetic tests are single-site testing, comprehensive single gene testing, and panel gene testing. More extensive genetic tests, such as exome (gene coding regions) and genome (gene coding and non-coding regions) sequencing, are also becoming more widely available.

Risks and Benefits The counselor will discuss the risks and benefits of genetic testing to help the parents make an informed decision about whether to have their child tested. The main reasons why parents decide to have their child tested are to help guide treatment and cancer screening decisions, to get an explanation for their child's cancer diagnosis, to determine whether the child is at increased risk for future cancers, and to clarify the risk of cancer for siblings and/or other relatives. In a study looking at parental attitudes toward testing their minor children for FAP, researchers found that the main barrier to genetic testing was the cost of the test [45]. Another barrier to testing may be the timing of the encounter with some parents feeling too overwhelmed or distressed about the child's diagnosis (or relapse) to consider genetic testing. In the United States, parents may also raise concerns about potential health insurance discrimination if the child were to test positive. However, parents can be reassured that this is currently unlikely to occur due to the Genetic Information Nondiscrimination Act (GINA) [46] and Health Insurance Portability and Accountability Act (HIPAA).

Potential Emotional Outcomes The psychological and emotional aspects related to genetic testing are also important to explore prior to testing. The family's experiences with cancer and perceptions about cancer risk often impact decisions about genetic testing and may increase baseline sadness and exacerbate cancer-related anxieties [43, 47]. The counselor can explore how these perceptions of risk might change based on different possible test results. Psychosocial benefits of testing can include an increased sense of control and empowerment. Potential risks of testing include heightened cancer worry, guilt, and symptom hypervigilance. The process of genetic testing of the siblings of a child with cancer might be fraught with additional distress for the children or their parents. Despite these challenges, families have generally described positive experiences and benefits of genetic testing and the genetic testing process [2]. Exploring additional questions such as "How do the parents envision reacting to the results?", "How have they coped with learning difficult news in the past?", or "What types of support do they have?" provides anticipatory guidance and can help parents decide whether they are ready to pursue testing.

Making Decisions About Testing Some families clearly know what they want to do, while others have a more difficult time making decisions about testing. Unless medical decisions are on hold, pending the genetic test results (a rare situation), the timing of testing is left up to the family. The counselor can explore with the family whether this seems like a good time to pursue testing and whether they would be able to cope with a positive test result. Parents can be given the option of deferring testing until the child's treatment has been completed or when the family is emotionally ready to proceed.

14.7 Possible Genetic Tests and Types of Results

14.7.1 Types of Genetic Tests

The types of genetic tests include:

Single-Site Tests Single-site tests are ordered when a specific variant has been previously identified in a relative, usually a parent or a sibling. The laboratory will evaluate only for the presence or absence of that single variant in a gene. It is important to have a written copy of the relative's result to send to the laboratory to ensure that the correct region of the gene is analyzed.

Single Gene Tests Single gene tests are often done when there is high suspicion for one particular genetic syndrome. Single gene testing should include sequencing and also deletion/duplication analysis. For example, a child with bilateral cystic nephromas would be offered *DICER1* testing, because *DICER1* syndrome is the most likely genetic syndrome associated with this particular finding.

Panel Gene Tests Genetic testing through multi-gene panels is now the most common testing method. It allows for the simultaneous testing of multiple genes on a single sample and is more cost-effective than other methods. Some syndromes are associated with multiple causative genes, making a gene panel a more efficient approach to genetic testing. For example, an adolescent with a pheochromocytoma can be offered a 12-gene panel to test for PGL-PCC syndrome, von Hippel Lindau syndrome, and multiple endocrine neoplasia type II. Gene panels may be focused, including only the genes specific to a certain cancer/tumor type, or they may be broad, including genes linked to a variety of cancers. In the adult setting, it is common to offer all panel options. However, there is ongoing debate in the pediatric cancer setting as to whether it is appropriate to offer broad panels which may include genes linked to adult-onset cancer risks [48, 49]. Other considerations of broad panel testing include the higher likelihood for detecting one or more variants of uncertain significance and the possibility of finding a P/LP variant in a newer gene with less information known about it. However, despite these potential issues, many pediatric cancer programs recommend that all patients be offered multi-gene panel testing regardless of the patient's cancer diagnosis, family history of cancer, or a known P/LP variant in a relative.

Whole-Exome Sequencing (WES) and Whole-Genome Sequencing (WGS) WES analyzes all exons (coding DNA within genes), and WGS analyzes exons, introns (non-coding DNA within genes), and non-coding sequences between genes. At the current time, most WES and WGS tests are performed as part of research studies. When ordered clinically, providers should be aware that laboratories limit the variants/genes reported to those associated with the patient's phenotype. However, the American College of Medical Genetics and Genomics has issued a list of genes, which, if containing a P/LP variant, should be reported back to patients regardless of the reason for referral [50].

WES and WGS testing will potentially be able to provide genetic diagnoses for the many families who have suspected genetic syndromes but for whom single gene or panel testing has been uninformative. However, learning how to interpret the vast quantities of data will take much time and effort. Testing will yield thousands of inconclusive VUS results which may be anxiety-provoking for both patients and providers. WES and WGS tests will undoubtedly become a common approach for clinical cancer genetics evaluation in the future and may replace single gene and panel testing options.

14.7.2 Types of Germline Genetic Test Results

The different types of results which can be obtained through germline genetic testing is shown in Table 14.4.

Table 14.4 Types of possible germline genetic test results and their implications [15, 51, 52]

Pathogenic (P) variant[a]: The child has a germline variant, and there is sufficient evidence that it affects the function of the gene and is associated with the hereditary cancer syndrome. This is considered a **positive result**
Likely pathogenic (LP) variant: The child has a germline variant, and there is evidence suggesting that it affects the function of the gene and is associated with the hereditary cancer syndrome, but the data is not strong enough to call it pathogenic. This is typically treated similar to a **positive result**
Variant of uncertain significance (VUS): The child has a germline variant (often a simple substitution of one DNA nucleotide), for which there is not enough information to classify it as pathogenic or benign. This is considered an **inconclusive result**
Likely benign (LB) variant: The child has a germline variant, and there is evidence suggesting that it does not affect the function of the gene and is not associated with the hereditary cancer syndrome, but the data is not strong enough to call it benign. This result is typically not present on a clinical report and is treated similar to a **negative result**
Benign (B) variant: The child has a germline variant, and there is sufficient evidence that it does not affect the function of the gene and is not associated with the hereditary cancer syndrome. This result is typically not present on a clinical report and is considered a **negative result**
No or B/LB variant, indeterminate negative: No P/LP variant was present on a clinical report. These results do not preclude the possibility of a missed P/LP variant in a gene analyzed or not analyzed
No or B/LB variant, true negative: This result means that the child does *not* have the germline P/LP variant present in the family

[a]Please see the text for additional information about these genetic test results

Pathogenic and Likely Pathogenic Variant (Positive Result) A positive test result means that a P/LP variant has been identified in a cancer susceptibility gene. Thus, the child has been diagnosed with a specific hereditary cancer syndrome. A positive genetic test result provides a diagnosis, guides future cancer surveillance recommendations, and, in some cases, influences the management of the current cancer. A positive result may also have implications for other relatives, including the child's siblings, parents, and other extended relatives. A small percentage of positive results are de novo (i.e., not inherited from a parent, appearing for the first time in that child), although germline or gonadal mosaicism should also be considered. The counselor will discuss the inheritance pattern of the P/LP variant and can discuss and arrange targeted genetic testing for at-risk relatives. It is important to note that as more data is accumulated, variants can change classification to higher or lower pathogenicity.

Example Justin, a 3-year-old diagnosed with a rhabdomyosarcoma of the maxilla, undergoes comprehensive *TP53* testing, and a P variant is detected. This positive *TP53* result means that Justin has LFS. As the oncology team develops his treatment plan, they will try to minimize the use of radiation, given the association of radiation and second cancer risk in people with LFS. Justin will also need to have lifelong cancer monitoring because of the increased risk of diverse cancers. In terms of the family, Justin's parents and older sister are offered single-site *TP53* testing with the understanding that they, too, might need increased cancer surveillance.

Variant of Uncertain Significance (Inconclusive Result) A VUS is a variation in the gene for which it is unclear whether it is associated with an increased cancer risk and is a P/LP variant (positive result) or it is simply a B/LB variant (negative result). Once the lab has gathered sufficient information on the VUS (a process that can take years), the VUS will be reclassified as either a positive or negative result. Most VUS results are ultimately reclassified as negative results. However, until the VUS is reclassified, screening recommendations are typically based on the child's personal and family history as well as the a priori probability of a positive result. VUS results can be challenging for both families and providers.

Example John, who had a soft tissue sarcoma at age 12 and also has a paternal uncle with a malignant brain tumor at age 45, underwent *TP53* testing. The *TP53* test identified no P/LP variants but did find a novel VUS. The VUS occurs in a region of the gene which is conserved through evolution; however, the predicted amino acid change is mild. The counselor disclosed the inconclusive *TP53* result to the family and explained that its current meaning was not yet known. The family was counseled to wait until further information was known about the VUS before testing John's unaffected siblings. John was advised to undergo medical follow-up based only on his personal and family history of cancer and not to receive LFS-related screening. The family enrolled in the lab-based family study which determined that the child's father carries the *TP53* VUS, but the paternal uncle does not. Therefore, the *TP53* VUS does *not* segregate with the cancer in the family. Three years later, John's *TP53* VUS result was reclassified as a benign variant (negative result), and no further genetic testing or additional cancer screening was indicated for the family.

Likely Benign, Benign Variant, or No Variant (Indeterminate Negative) A comprehensive test that identifies no P/LP variants or VUSs is termed an indeterminate negative result. This type of result means that the child does not have a detectable P/LP variant in the tested gene(s). However, the family needs to be cautioned that a hereditary susceptibility cannot be completely ruled out. If the child had only a small likelihood of having a cancer syndrome, then this negative result is reassuring. Conversely, if the child had a high risk of having a cancer syndrome, then further genetic testing may be indicated. It may also be reasonable to recommend additional cancer surveillance based on the child's personal or family history.

Example Maria was diagnosed with ALL at age 4. Her mother is cancer-free at age 40, but the maternal grandmother had breast cancer at age 65. Maria's *TP53* test revealed no variants. This negative result is reassuring that Maria does not have LFS, because of the low a priori likelihood of a *TP53* P/LP variant, and no additional genetic testing or cancer screening would be necessary. However, if Maria had ALL and her mother and grandmother had both developed breast cancer before age 35, then the family would have a much higher a priori likelihood of having LFS. In this case, a negative *TP53* result would not be as reassuring, and additional genetic testing and possible cancer screening would be considered.

Likely Benign, Benign Variant, or No Variant (True Negative) A true negative result means that the person does not carry the specific P/LP variant that is present in the family. To obtain this type of result, the specific P/LP variant in the family needs to have been previously identified. Since the child does not have the variant known to be in the family, he/she is not considered at increased risk for the cancers associated with it and does not need any additional cancer surveillance.

Example Hannah, who is cancer-free at age 10, undergoes single-site testing to look for the *TP53* LP variant recently identified in her younger brother. This test determines that she does *not* carry the *TP53* variant. This is a true negative result which means that Hannah does not have LFS and does not need any special cancer screening. Her cancer risks are likely to be the same as children in the general population. In addition, Hannah's future children will not be at risk for inheriting the *TP53* LP variant and will not need to be tested since Hannah does not have it, herself.

14.8 Posttest Counseling Discussions

Genetic test results are typically reported directly to the child's parents, as well as to his/her oncologist or referring physician. These results can be disclosed in an in-person visit or by telephone, with follow-up visits scheduled as necessary. The result disclosure can invoke a range of reactions from the parents. Although most people cope well with the news over time [53, 47], the involvement of children is likely to compound the emotional response. For this reason, many parents wish to learn the result without the child being present. However, some adolescents may wish to be included in these discussions.

Discussions about the genetic test results are often guided by the parents and child. The counselor will stay attuned to the family's reactions during the discussion to avoid overburdening them with information. At this visit, the counselor is typically joined by a geneticist or pediatric oncologist who can focus on the medical issues and questions.

The following topics often come up during discussions of positive test results:

Review of the Result The family will be provided with an explanation of the meaning of the genetic test result. This includes information about the cancer syndrome, associated cancer risks, and the accuracy and limitations of the result. The counselor will explain how to read the test report and will usually give the parents a copy of the report for their records. At most centers, a copy of the genetic test report is placed in the child's medical record.

Medical Recommendations Based on the test result and the child's personal and family history, the medical provider will discuss the recommended regimen for future cancer surveillance, arrange for appropriate screening tests, and make refer-

rals to other specialists as needed. The provider will also work with the child's oncology team if the result has implications for the child's current treatment plan.

Implications for Relatives The counselor will review the risks to other relatives. Most hereditary cancer syndromes are autosomal dominantly inherited, meaning that there is a 50% risk to first-degree relatives (siblings, parents, and future children) and a possible 25% risk to second-degree relatives (aunts, uncles, and grandparents). One of the first steps following the identification of a P/LP variant in a gene is to test the parents to determine which side of the family is at risk. If neither parent carries the gene P/LP variant, then none of the second-degree relatives will need to be tested; however, the child's siblings should be offered testing due to the possibility of germline or gonadal mosaicism. The counselor can help the parents disseminate the information to other relatives by writing a family letter and offering to speak to the relatives directly. The counselor can also arrange genetic testing for the relatives or can make referrals to local testing programs.

Future Family Planning The counselor will also address the option of reproductive genetic testing options, including prenatal testing or pre-implantation genetic diagnosis. The counselor can refer the family to a fertility specialist if they wish to learn more about these options. For a variety of reasons, this may or may not be relevant to the family; however, it is important that the family be aware that such options do exist.

Coping The counselor will also check in with the family regarding how everyone is adjusting to the news and to ascertain whether any additional support is needed. Limited information is available about the short- and long-term impact of receiving positive genetic test results during childhood or adolescence. One study showed that adolescents and young adults believe there are important benefits to genetic testing, such as reducing uncertainty and anxiety by better understanding cancer risks and engaging in risk-reducing or preventative interventions and behaviors [54]. In adulthood, this news may bring an increase in cancer-related worries. One study found that adults without a cancer diagnosis, who tested positive for a cancer syndrome, had increased uncertainty regarding their future health risks, similar to that of individuals with cancer [55]. Parents of children who tested positive for FAP and MEN2 experienced increased levels of depression [56] and increased levels of both generalized and MEN2-specific anxiety, in comparison with parents whose children tested negative [57], respectively. The genetic counselor may make referrals to mental health providers and patient or family support groups, which can be invaluable resources during the coping process.

Need for Follow-up Visits Patients who test positive for a hereditary cancer syndrome or are suspected of having one are encouraged to be evaluated on an annual basis in a cancer genetics specialty clinic. This allows families to obtain the most up-to-date information about the syndrome, the associated cancer risks, and the recommendations for cancer risk management. It also provides families with the opportunity to address questions or concerns that may arise over time. This may

include how to discuss the test results with the child or what screening is recommended as he/she becomes older.

14.9 Special Genetic Counseling Issues

All individuals enter genetic counseling with a particular "lens," which influences their perception of risk, their ability to process information, and their manner of coping with genetic information. This lens is shaped by multiple factors including the family's experiences with cancer, their ethnic culture, religious and spiritual beliefs, level of education and cognition, age and maturity, personality traits, and coping mechanisms [58]. Although certain genetic counseling issues are encountered across all specialties, these issues may be intensified when dealing with children who have cancer.

The most common counseling challenges encountered in pediatric cancer counseling are described below:

Multiple People in the Room The genetic counseling sessions may be attended by several individuals, including the child and his/her parents, as well as the child's siblings, aunts, uncles, grandparents, and even friends of the family. It can be challenging to provide information and support amidst complex family dynamics and competing agendas. For example, raising the option of genetic testing may invoke different reactions in the family, from the child who dreads having another blood test to the parents who feel they "have to know" the genetic test results even though it won't impact treatment and the grandparents who are opposed to testing because they fear learning that one of them passed on a faulty gene to the child.

Involvement of the Child Although the parent/guardian provides authorization for testing, the child may be involved in the testing process to varying degrees, depending on the child's age (typically age 10 or older), maturity level, and the ability to think abstractly in order to provide assent. The genetic counselor will provide age-appropriate information about the test and focus on the topics most relevant to the child or adolescent in the immediate future. Involving children or adolescents in the decision-making process requires a delicate balance between providing them with meaningful information and making sure they do not become overwhelmed by the discussion. Children and adolescents may be influenced by their parents' attitudes regarding genetic testing and cancer monitoring and whether they tend to agree with or rebel against their parents' wishes. They may also have special concerns about the test which need to be recognized and addressed [41, 39].

Example Kayla, age 12, and her parents meet with a genetic counselor to arrange single-site *SDHB* testing. Kayla is cancer-free, but her father has had two paraganglioma tumors removed and carries an *SDHB* LP variant. Although Kayla assents to the test, the counselor notes that she seems quite worried about it. The counselor arranges to talk to Kayla privately prior to the blood draw. After a bit of encourage-

ment, Kayla asks the counselor if a negative test result would mean that her father is not really her father. The counselor assures her that this is *not* the case; her father could have given her an altered copy of the gene *or* a normal copy of the gene. Greatly relieved, Kayla has her blood drawn for testing.

Separated or Divorced Parents When a child's parents are separated or divorced, it can add a layer of complexity to the counseling and testing process. Parents who are separated or divorced may not be equally informed or involved in the testing process. The counselor may need to have separate conversations with each parent to ensure that both of them have the information about testing and have a voice in the decision-making process. The counselor also needs to abide by the custody agreement in terms of who can authorize genetic testing.

Example Connor was recently diagnosed with hepatoblastoma at age 2. Connor's parents are divorced, and although they have shared custody, they have limited contact with each other. The counselor meets with Connor's mother to discuss *APC* genetic testing. The mother wants to pursue testing and a separate testing appointment is scheduled. Connor's mother requests that the counselor call the child's father and explain the testing process to him. The counselor speaks at length with Connor's father, who expresses some concerns and reservations about the test. Following this discussion, he agrees that Connor should be tested at some point, but he requests that testing be deferred until after the child's treatment is completed. The counselor conveys this request to the child's mother who ultimately agrees with this plan, and the testing appointment is rescheduled for a later date.

Children Who Are Wards of the State or in Foster Care Guardianship issues may also pose a significant challenge. For foster children who are wards of the state, medical and genetic testing decisions have to be authorized by a third party who may not understand all of the issues at hand. In addition, the guardian may have limited information about the child's biological relatives, making it difficult to assess the child's risk for a cancer predisposition syndrome. Limited contact with biological relatives also poses an obstacle to informing other potentially at-risk family members if the child tests positive for a gene variant. Another concern is the stigmatization or labeling of a child whose potential adoptability may be impacted by the increased cancer risks or need for lifelong screening [59].

Example Henry underwent surgery at age 4 to remove a medullary thyroid cancer. Henry has been a ward of the state since he was an infant. He has no information about his father and has limited contact with his mother. Henry, his caseworker, and his foster parents meet with the counselor to arrange *RET* genetic testing. Henry tests positive for a *RET* P variant, and the counselor and oncologist discuss the features of multiple endocrine neoplasia type II and the need for follow-up monitoring. The counselor also talks about the importance of offering predictive *RET* testing to Henry's birthmother and maternal half siblings (all in foster care). With the help of the caseworker, the counselor is able to contact the birthmother and the other foster families to discuss the option of genetic testing.

Children with Terminal Illness Parents of terminally ill children may request testing to clarify the risks of cancer for other family members. However, counseling while a family is grieving is difficult for everyone involved. The parents may be interested in learning the child's genetic test results but may have a diminished capacity to "hear" the news and to process the implications for themselves and their other children. The parents may also hope that a positive genetic test result could give the child further treatment options, which unfortunately is rarely the case. It is important that families have reasonable expectations regarding the genetic test. Some families may decline testing because they do not want to subject the child to additional discomforts (e.g., needle sticks for blood samples), especially if there are no immediate implications for his treatment or prognosis. The counselor can remind parents that other people in the family could pursue genetic testing either now or in the future, although testing the child with cancer may be the most informative person in the family. And lastly, the counselor can raise the option of DNA banking so that the family would have the option of pursuing genetic testing at a future time [60].

Children from Other Countries Parents with children who have traveled to the hospital from another country may have the added layer of language and cultural barriers to the already complex and emotionally intense subject matter. In addition, the family's attitudes toward testing and westernized medicine, their perceptions of cancer risk, and their decision-making styles may differ greatly [61]. For example, in some cultures, decisions about medical care, including decisions about genetic testing, are made by the head of the household, who is not necessarily the patient's parents. Other aspects of the testing process may also be more complex, including the need for an interpreter at the counseling sessions, fewer written resources to offer the family, and the difficulty in determining whether and how the genetic testing costs will be covered.

Children in Economically Disadvantaged Families Children in economically disadvantaged families may have less access to genetic counseling and testing services due to poor insurance coverage and the inability to pay out of pocket for these services. The counselor will work with the family to try and get these services covered. However, for some families, the cost of genetic testing remains prohibitively expensive. As the cost of genetic testing decreases, hopefully, this will become less of a barrier to genetic counseling and testing over time.

14.10 Case Examples

14.10.1 Case 1: Family History of Known Cancer Syndrome

Jane is a 35-year-old woman who has FAP due to an identified *APC* P/LP variant. She had a colectomy at age 20 due to adenomatous polyposis. She also has multiple fundic gland polyps and duodenal adenomas. Her father also has FAP with a history

of colectomy following colon cancer and desmoid resection. Jane has three children who are 6, 10, and 13. She and her husband understand that each child has a 50% chance of inheriting the familial *APC* P/LP variant, and they are interested in having them tested.

Jane, her husband, and all three children were referred for genetic counseling and testing. The children are all healthy with no current gastrointestinal problems. None of them have had a sigmoidoscopy or colonoscopy. During the initial visit, the parents met first with the counselor and physician to discuss the clinical aspects of FAP and management and to discuss how best to talk to the children about the test. The children were then brought into the room to discuss the test and address questions. Brief information was discussed at their age-appropriate level, and the two older children were asked to provide assent for the test. The youngest child was scared of getting his blood drawn and initially hid under the conference room table. However, he was eventually coaxed into sitting on his father's lap. The three children had their blood drawn for testing at the end of the visit, and the counselor arranged to call the parents with the results and also scheduled a follow-up visit for the family.

One month later, the family returned to the clinic. The two youngest children tested positive for the familial *APC* P/LP variant, while the eldest tested negative. Jane was devastated by the news; she was hoping that all three would test negative. She expressed parental guilt for passing on the disease burden to her children. Her husband was very supportive and glad that they knew what the risks were so that they could prevent cancer. The children who tested positive did not seem to be overly distressed by the results, but the youngest was afraid of getting more blood drawn. The eldest, who tested negative, was very mature for her age (13 y) and expressed survivor's guilt. She stated that she wished she had been the one to have the *APC* P/LP variant, because she knew she could have handled it better than her younger siblings. The family also met with the pediatric gastroenterologist to discuss when the initial colonoscopies would be performed for the two children who had tested positive, and the family was also referred to the program psychologist to provide support as needed.

14.10.2 Case 2: Diagnosed with Cancer Suggestive of Hereditary Cancer Syndrome

Sara is a 2-year-old girl who was recently diagnosed with a rhabdomyosarcoma of the proximal tibia. She has two healthy brothers, ages 4 and 7. Sara's parents are both in their 40s and have never had cancer, nor have their siblings. Sara's paternal grandfather had two basal cell carcinomas removed in his 70s. There were no other cancers reported in the family.

Sara's doctors referred Sara and her parents for genetic counseling and testing. During the visit, the genetic counselor offered *TP53* testing, and Sara's risk for carrying a P/LP variant was estimated to be 5–10%. Given the parents' acute distress over Sara's cancer diagnosis, the genetic counselor offered to postpone testing if

they preferred. However, Sara's parents insisted on having her tested that day, as they felt the information from the results would be important for Sara's medical management. They were also clearly hoping for "good news," i.e., a negative test result. The parents and the counselor agreed to meet during Sara's next treatment visit for the disclosure of the results.

About 1 month later, the genetic counselor received the laboratory report indicating that Sara had tested positive for a *TP53* P variant. She met with the family to disclose these results, and they were understandably devastated by the news. Sara's oncologists were now wary of treating Sara's cancer with radiation, given her positive *TP53* status. Sara's parents were extremely distressed about what this meant for Sara's treatment plan and her future cancer risks. They were also very concerned about their other children's cancer risks. Sara's parents and brothers were all tested for the *TP53* P variant identified in Sara, and thankfully all of them tested negative. The counselor explained that this meant that Sara's *TP53* variant was most likely a de novo genetic event, although Sara's future children will have a 50% risk of having the *TP53* variant. Sara's parents obtained second and third opinions about her cancer treatment options in light of the *TP53* variant. Ultimately, she underwent resection with proton beam radiation therapy and is now doing well.

14.11 Conclusion

Genetic counseling has been defined as "the process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease" [1]. The purpose of this chapter was to provide an overview of the genetic counseling and testing process and to illustrate the complexities and challenges that can arise in the provision of pediatric cancer genetic counseling.

Genetic counselors have specialized training and expertise to provide information and support to families who are at risk for having a hereditary cancer syndrome. Currently there are a small but growing number of programs offering pediatric cancer genetic counseling. The National Society of Genetic Counselors website provides information and resources about genetic counseling and also is an excellent resource for identifying local genetic counselors within and outside of the United States [62].

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Chapter 15
Psychosocial Aspects of Childhood Cancer Genetics

Andrea Farkas Patenaude and Claire E. Wakefield

Abstract Recent advances in genomic technologies have enabled increasing identification of children who carry a germline pathogenic variant in a cancer predisposition gene. This development has led to increasing numbers of children being offered cancer-related genetic or genomic testing. Early identification of an underlying cancer predisposition syndrome may influence treatment approaches for children with cancer, as well as guiding longer-term surveillance and risk reduction for at-risk children and their family members. Despite this exciting potential, there is little data available on the short- and long-term impacts on children and their families. In this chapter, we review the available evidence regarding the psychosocial impact of genetic testing on children and their families while also summarizing current research on family attitudes toward genetic testing, the impact of surveillance, and any influences on reproductive decision-making. The chapter focuses on families affected by Li-Fraumeni syndrome, familial adenomatous polyposis, retinoblastoma, von Hippel-Lindau syndrome, and multiple endocrine neoplasia type 2. The chapter also addresses recent innovations, such as the adoption of precision medicine, and explores their potential impacts on well-being. We present recommendations for providing tailored psychosocial support to families, as well as offering guidance for future rigorous psychosocial research. Understanding more about the psychosocial aspects of childhood cancer genetics will be essential in enabling us to determine the impact of advancing technologies and to provide effective psychosocial support to vulnerable children and their families.

Andrea Farkas Patenaude was deceased at the time of publication.

A. F. Patenaude (Deceased)
Dana Farber Cancer Institute, Boston, MA, USA

C. E. Wakefield (✉)
School of Women's and Children's Health, UNSW Sydney, and Kids Cancer Centre, Sydney Children's Hospital, Randwick, NSW, Australia
e-mail: c.wakefield@unsw.edu.au

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15.1 Introduction

"Maybe there are some things you just shouldn't study." Such were the words of a senior genetics advisor at a meeting of the National Institutes of Health (NIH) Ethical, Legal and Social Implications (ELSI) Advisory Board in the early days of genetic discovery in the late twentieth century about a proposal to include children in the first-ever study of genetic testing of Li-Fraumeni syndrome family members. The statement embodied both the serious concern at that time about whether personal knowledge of genetic test results for hereditary cancer predisposition might be psychologically devastating and a desire to protect children, even if it meant excluding them from research.

Study of the impact of cancer genetic testing on individuals in hereditary cancer families has been greatly enhanced in the USA by the early decision to devote 5% of the budget of the Human Genome Project to the study of resulting ethical, legal, and social implications [1]. We know now that our early concerns about genetic test results causing clinical levels of anxiety and depression or even suicidal ideation were overly pessimistic [2]. Knowledge of the impact of hereditary cancer and genetic testing on adults from families affected by *BRCA1/2* and Lynch syndrome has grown substantially [2–6]. Our understanding, however, of how children in families affected by hereditary cancer syndromes are impacted remains limited [2]. This is especially true for syndromes which predispose to cancer in childhood. Studying psychosocial outcomes of genetic testing and screening in children at risk for hereditary syndromes has intrinsic complications and challenges, but research is essential so that we can better understand how young people incorporate and cope with hereditary cancer risk and with potentially life-saving screening options. We will review the intrinsic issues and data from studies of children in families affected by Li-Fraumeni syndrome (LFS), familial adenomatous polyposis (FAP), retinoblastoma, von Hippel-Lindau syndrome (VHL), and multiple endocrine neoplasia type 2 (MEN2). We will also discuss how recent changes in the way some children are screened, and changes in the nature of genomic testing, may affect the future psychological well-being of parents and children in hereditary cancer families.

15.2 What Is Different when the Diagnosis of Cancer in a Child Involves Identification of a Hereditary Cancer Syndrome?

Diagnosis of cancer in a child is many parents' worst nightmare [7, 8]. It arouses intense fear, challenges beliefs about being able to provide a safety net for children, and opens up a foreign world of hospitals, risk statistics, and burdensome and frightening treatments. Many parents and children feel singled out and worried about what they might have done wrong to be confronted with the challenge of cancer in a child [9, 10].

When, along with the diagnosis of cancer in a child, that child is also identified, through genetic testing and/or family history, as having a hereditary cancer syndrome, the impact on the family is magnified many times [11]. Table 15.1 summarizes the ways in which diagnosis of a hereditary cancer syndrome in a child with cancer can amplify parental worry. In most pediatric cancer diagnoses, parents can be reassured that their other children are unlikely to be at increased risk of developing cancer. When the child who has cancer is also found to have a genetic mutation which is then found also in a parent, it becomes clear that each child of that parent has an increased risk of carrying the same deleterious mutation and, therefore, has a higher than average chance of developing cancer. Increased cancer risks may be present in the parent as well. Members of the extended family need to be informed, and they, too, may be found to be mutation carriers, enlarging the circle of fear and worry [11]. Experts in the treatment of hereditary pediatric cancer syndromes may need to be found, often at great distance from where the family lives or where the child is treated. Management decisions may change based on expert advice from pediatric oncologists with specialties in cancer genetics or from pediatric genetics experts. Some of these decisions may be different to treatment decisions made for a child with a "standard" case with the same disease without the hereditary component (e.g., children with hereditary syndromes may be recommended to avoid radiation treatment due to their increased risk). The child's greater risk of developing second cancers, as is often the case in hereditary syndromes, may make parents realize that even cure of the current cancer will leave them worried about the child's future health [11]. Lifelong surveillance may be necessary, often looking not only for early signs of multiple forms of cancer but also, as is frequently necessary, for nonmalignant (but not necessarily nonlethal) diseases which are part of the syndrome. Parents can feel overwhelmed by the fears they have about multiple family members and may also feel guilty if they are the parent who conveyed the deleterious mutation to their child [11, 12]. Given these concerns, it is clear that psychological data on parents of children with hereditary cancer syndromes and on the children themselves is of great interest and potential benefit in planning for and organizing the treatment of these children and their family members.

Table 15.1 What difference does hereditary etiology make in a pediatric cancer diagnosis?

Child diagnosed with cancer: nonhereditary etiology	Issue	Child diagnosed with cancer: hereditary etiology
Only this child; parents generally reassured about sibling risk	**Diagnosis affects cancer risks for…**	Child and potentially siblings, parents, cousins, aunts, uncles, grandparents, future offspring
Clinical assessment of child	**Diagnosis based on…**	Clinical assessment of child plus family history and genetic testing
At all comprehensive cancer centers treating pediatric patients	**Medical expertise**	Experts in treatment of hereditary pediatric cancer syndromes are scarce
Clinical assessment of child and tumor, including genetic analysis of tumor	**Management decision based on…**	Clinical assessment of child and tumor, including genetic analysis of tumor plus possible information from genetic analysis of germline
Variable depending on tumor type and treatment received but typically low	**Second cancer risk**	Variable, but often significantly increased (e.g. could be 50% or more in some syndromes); also may be at increased risk for serious "benign" tumors
In general, decreasing risk of recurrence with time. Clinical follow-up recommended with less frequent visits and/or less aggressive surveillance over time	**Surveillance recommendations**	Often lifelong surveillance required for increased cancer risks and other possible medical diagnoses associated with the syndrome
Challenging to find adult providers knowledgeable about pediatric cancer survivors	**Transition to adult providers**	Few experts knowledgeable about adult management of pediatric cancer syndromes
Possible infertility. If fertile, it can be reassured that cancer risks to offspring are low	**Reproductive issues**	Possible infertility. If fertile, there may be as high as a 50% risk of passing on the inherited gene alteration to offspring. Pre-implantation genetic diagnosis or prenatal diagnosis may be considered
Devastating, but coping improves over first several months post-diagnosis if child does well	**Emotional impact-parents**	Devastating. Genetic diagnosis may make it harder to cope with child's diagnosis and long-term health management.
Can be reassured the diagnosis is not their fault	**Guilt-Parents**	Can experience feelings of guilt if the inherited cancer risk has come from one parent
Well-documented initial fears followed by adjustment to treatment. Most show resilience	**Emotional impact- Child**	Unknown. Little child self-report data in most pediatric cancer syndromes
Possible feelings of isolated from peers. Can find others in hospital and clinics with same diagnosis, treatment	**Isolation-Child**	Possible feelings of isolations from peers. Unlikely to find others in hospitals or clinics with same diagnosis/syndrome

15.3 Challenges in Studying Children with Hereditary Cancer Syndromes

Several factors have limited the amount or generalizability of evidence amassed about psychological reactions and adaptation among parents of children with hereditary cancer syndromes and, particularly, among affected children. Pediatric cancer is rare, with approximately 11,050 cases per year in the USA expected for 2020 [13]. The number of children found each year to also have a hereditary cancer syndrome is a small fraction of these cases, though, with increasing genetic and genomic knowledge, the number of cases identified has increased in the last decade [14–16]. Even at the largest pediatric cancer centers, there may be months or years between the presentations of cases with the same hereditary cancer syndrome. Thus, research on the medical or psychological characteristics of children with hereditary cancer syndromes necessitates multi-site cooperation and collaboration. Given that small absolute numbers of children with the same syndrome may be able to be enrolled on a particular study at any one time, funding can also be more difficult to attract. Registries, which would be helpful in accessing children with hereditary cancer syndromes, are expensive and difficult to establish and maintain and require careful ethical oversight, although collaborative approaches to the development of child cancer predisposition syndrome registries are emerging in some countries.

There are also measurement problems which complicate this work. There is no clear agreement about what particular psychological issues and factors are most important to study in hereditary cancer families and which measures or even methods are best suited to gather this psychological data [2]. Qualitative data in a relatively novel field of inquiry is often desirable but may be open to claims of subjectivity [17]. Measures of quality of life developed for children with cancer may not capture some of the particularly distressing aspects of hereditary cancer, such as the fact that significantly increased cancer risks continue for not just the patient but for other family members as well. Assessment of children at markedly different ages, which is likely to be needed to capture all in the small cohort of children with hereditary cancers, may necessitate testing children over a broader developmental spectrum, further complicating and limiting the choice of appropriate instruments. Inclusion of several family members or members of closely related families in hereditary cancer studies also necessitates statistical correction to account for these interrelationships.

15.4 What Are the Critical Psychosocial Questions?

Psychosocial studies which are reviewed in this chapter focus on one or more of the following critical questions concerning psychological issues in hereditary cancer families (Table 15.2).

Table 15.2 What are the important psychosocial questions?

1. What is the nature and extent of cancer-related distress in members of hereditary cancer families, parents, and children?
2. What factors predict this distress?
3. What is the psychological impact of genetic counseling and testing on children?
4. What is the uptake of genetic testing?
5. To what extent are screening recommendations followed?
6. What are the psychological ramifications of screening in affected and unaffected children?
7. What interventions are useful in diminishing distress and increasing uptake of recommended screening or surgery?
8. What is the psychosocial impact of new technologies on children and their families, including direct-to-consumer testing, whole-genome sequencing, and precision medicine?

15.5 Li-Fraumeni Syndrome (LFS)

Li-Fraumeni syndrome (LFS), one of the first pediatric cancer syndromes to be defined [18], involves the development of malignant tumors during both childhood and adulthood. Penetrance is very high, with over 90% of carriers experiencing at least one and often several primary cancers [19, 20]. Females have higher rates of cancer than males, and cancers tend to occur earlier in female versus male mutation carriers [21]. Up to one quarter of *TP53* mutation carriers will develop cancer by age 20 [20, 22]. Up to one third of carriers will develop multiple primary cancers [19, 20].

Thus, the sword of Damocles (representing a sense of imminent peril) feels constant for LFS family members, and the experience of illness and loss in especially the originally identified, highly impacted families seems never-ending [12, 23]. Genetic counseling for LFS is considered a "lifelong process" [24]. When losses occur in quick succession in hereditary cancer families, it is often difficult for younger family members to differentiate themselves and their likely future path from that of ill or deceased relatives, increasing the likelihood of prolonged, unresolved grief [25].

Genetic testing for LFS, made possible by the 1990 discovery that mutations in the *TP53* gene were the primary cause of LFS [26], differentiates mutation carriers from non-carriers. Testing in research settings for *TP53* mutations in families affected by LFS began in 1994 and is now available in clinical laboratories. Early research showed that, as was the case for Huntington disease, though not so dramatically, the actual uptake of genetic testing for *TP53* mutations in LFS family members was initially considerably lower than what had been forecast prior to the cloning of the *TP53* gene. In the earliest study, 39% of adult members of LFS families in an LFS registry accepted genetic testing [27]. Since then, studies in the USA and in Europe have shown increasingly higher rates of uptake of genetic testing [28, 29].

We have not comprehensively studied quality of life and behavioral outcomes of the genetic testing of children in LFS families [2, 30]. Hence, we understand little

about what it means to grow up in an LFS family. At a meeting held at the US NIH in November 2010 about biological, medical, and psychological aspects of LFS, 60 members of LFS families attended, and several affected and unaffected family members offered vivid, detailed reports of the experience of living with LFS, which are reprinted in the meeting report [24]. These narratives contribute to our awareness of the complexity and the nature of the continuing distress regarding LFS felt by family members across the lifespan, but we are still in need of longitudinal interviews with children in LFS families to better understand what supportive services could help them cope [2, 30].

15.5.1 Psychosocial Outcomes of LFS in Childhood

Issues which have been identified for LFS families include considerations about identity and feelings of isolation [25]. There are many aspects of identity across the lifespan which are affected by being part of an LFS family, including a sense of having a family which has been singled out for "bad fortune," one with many more serious cases of cancer than most other families and multiple, early deaths [12]. In a study of 92 untested individuals in LFS families, 74% had experienced an LFS-related death, with 26% having experienced the loss of a sibling or parent before the age of 21 [29]. For many people in such families, a sense of doom predominates about cancer [12, 23]. Illness-related identity also plays an important role in considerations of dating, marriage, and childbearing for LFS family members.

Sharing with a potential significant other such a devastating life history is daunting, especially given the consequent potential risks for subsequent generations. Such feelings may initially make members of these families hesitate to plan to have children. However, young people in LFS families have also expressed that such feelings suggest a negative value to their own lives, affecting their own views on the use of reproductive technologies to avoid birth of an LFS-affected child. Some have said that they realized that if their parents had avoided having a baby with LFS, they would never have been born [25, 31]. Hence, many people who are LFS mutation carriers have opted to have children despite the cancer risks that could be passed on, while others feel it is imperative to avoid bringing a child into the world to face such risks.

A sense of isolation is common in LFS and other hereditary cancer families as the concerns about cancer are so much more prominent and pervasive than in most families [31]. Family members can find it difficult to talk to friends or colleagues, often feeling that friends or colleagues do not fully understand the magnitude of the risks they live with. Even within some LFS families, it is difficult to talk about hereditary cancer, especially with children [12, 23]. Talking may evoke grief related to deaths of family members from cancer or uncomfortable feelings between those found to be mutation carriers and those who either tested negative or who have not sought testing. Parents report that talking to children about LFS in a developmentally appropriate way is critical but also challenging [12]. There may also be

differences of opinion within the family about how open the communication should be to children about hereditary cancer or about whether prophylactic measures should be undertaken or how actively screening should be pursued [32].

There are few patient-reported studies of other psychosocial outcomes in children affected by LFS. In one small study with seven adolescents and young adults who had undergone *TP53* mutation testing, young people reported that the result had not significantly impacted their family relationships but impacted their plans for the future (e.g., career choices and family planning decisions) [33]. Young people also report worrying about experiencing feelings of fear, anger, shock, and disappointment should their test reveal that they are a *TP53* mutation carrier and sometimes report that they did not fully understand the lifelong implications of LFS until after they received a positive genetic test result [30, 33].

15.5.2 Genetic Testing for LFS in Childhood

A recent review revealed that most parents of children at risk for LFS have positive attitudes toward undertaking *TP53* mutation testing for their children, describing the potential medical and psychosocial benefits of early clarification of their risk [31]. Some parents express concerns about a positive *TP53* result, increasing their child's anxiety, reducing their children's future insurability, or diminishing their child's autonomy by making the decision to undergo genetic testing before their child can make the decision for themselves [31]. Parents also describe a sense of accountability and burden when making these long-term, difficult decisions for their children [12].

The few studies that have evaluated children's attitudes toward genetic testing for LFS and their experiences of a positive result have reported limited, if any, psychological difficulties after testing [31, 34]. Like their parents, adolescents and young adults from LFS families appear to have positive attitudes toward genetic testing, expecting that receiving a test result will reduce uncertainty and anxiety [33]. It is important to note that while families and young people often report positive attitudes toward *TP53* mutation testing in children, clinicians can be more hesitant about balancing the potential benefits of testing with potential long-term disadvantages for children, citing concerns about the impact of lifelong screening individuals for LFS [30, 35, 36].

15.5.3 Screening in Children with LFS

Research to consider the benefits of active screening of unaffected children in LFS families who carry *TP53* mutations appears to be changing the risk/benefit ratio regarding the genetic testing of children in these families. At the Hospital for Sick Children in Toronto, Malkin and his group initiated a comprehensive surveillance

protocol in asymptomatic *TP53* mutation carriers in LFS families, both adults and children, which yielded promising results in terms of identifying early-stage cancers and improving survival outcomes [37–39]. This research has spawned a number of other studies aimed at replicating these findings, with the goal of determining what surveillance practices should be recommended for child and adult *TP53* mutation carriers in LFS families to try to reduce the high morbidity and mortality. This work has the possibility of drastically changing the life experience and life histories of LFS family members [37], and it is, therefore, important that attention be paid to the psychological impact of lifetime screening beginning early in childhood for unaffected mutation carriers [39, 40].

15.5.4 Reproductive Decision-Making

Pre-implantation genetic diagnosis (PGD) offers couples an opportunity to avoid having a child with a known genetic abnormality which is present in one of the parents. Couples have to be willing (and financially able) to undergo in vitro fertilization. Embryos are tested at the 3-day (eight-cell) stage, and only those without the deleterious mutation are implanted into the mother's uterus [41]. The technique has been used by increasing numbers of couples in which one member is from a family with a hereditary cancer syndrome [41, 42]. There are ethical and psychological issues which couples and providers [41, 43] contemplating the use of PGD must consider, and, to date, the rates of uptake for PGD among hereditary cancer families have been low. A study from the Netherlands surveyed male and female high-risk individuals ($n = 179$) from LFS and VHL families and found that approximately half of those contemplating future pregnancies said that they would consider PGD, though the authors cite various reasons for believing this number is an overestimate of those who will ultimately use PGD [44].

There is not yet much data on how many couples utilize PGD or other prenatal genetic technologies to prevent the birth of a child with hereditary predisposition to a pediatric cancer syndrome. Because of the sensitivity of the issue, it may be difficult to acquire accurate statistics. Perhaps, the most important immediate concern is that information about such interventions be imparted to parents of currently affected children who are wanting to have more children and to individuals with inherited pediatric cancer syndromes approaching reproductive age to offer the option of having children, should they wish, who are free of the mutation responsible for the hereditary cancer in their family. This issue must be sensitively presented, however, as it raises issues about the value of the lives of those who do carry the deleterious mutation.

15.6 Familial Adenomatous Polyposis (FAP)

Familial adenomatous polyposis is a cancer syndrome necessitating colorectal screening in late childhood and adolescence and, when 100 or more colon polyps are found, prophylactic colectomy, typically occurring between the ages of 15 and 25 years. FAP is caused by mutations in the *APC* gene [45]. Without screening and subsequent surgery, 100% of FAP patients would be expected to develop colorectal cancer by age 40 [46]. Current guidelines recommend that predictive genetic testing be offered to children at risk of FAP around 12–14 years, or earlier if they are symptomatic [47]. Children with FAP are recommended to commence colorectal cancer screening between age 12 and 14, with recommended screening frequency tailored to the child's case [47]. There are small but growing patient-reported data about the psychological distress and attitudes toward screening and surgery of minor children in FAP families.

15.6.1 Psychosocial Outcomes of FAP in Childhood

There is a small body of literature examining the psychosocial impact of FAP on children and young people. Michie [48] studied 60 children, ages 10–16 years, from FAP families, 31 of whom were mutation carriers and 29 of whom were not. The children were assessed on several standard psychological measures with an average of 3–5 years after they had undergone genetic testing. The authors found depression, anxiety, and self-esteem scores were in the normal range on average and did not increase over time, but there was a trend for depression and anxiety scores to be higher among children who had tested positive than those who had tested negative [48]. The mutation carriers worried more about FAP, perceived their cancer risks as higher, and felt more threatened than children testing negative. Children who were not carriers had distress scores below US norms. The great majority of the children, positive and negative, reported good health status. Children who had tested positive were less anxious than adults who had tested positive for FAP [48].

At a mean of 38 months post-genetic test disclosure (range 23–55 months), Codori [49] found that among 48 children tested for FAP (approximately half were positive and half true negatives), all group means on measures of depression, anxiety, behavior problems, and behavioral competence were within normal limits. Having a mutation-positive sibling, however, significantly increased depression among similarly mutation-positive brothers or sisters and tended to increase anxiety in mutation-negative brothers or sisters. The authors concluded that "it would be clinically irresponsible to ignore the increases in depression and anxiety among children with positive siblings" and suggested that emotional support be provided to families where children had mixed genetic test results.

A higher degree of psychiatric disturbance was found in a Norwegian study of 22 teenagers with a parent with FAP, who were interviewed and assessed on standard

psychometric measures [50]. Subjects were ages 11–20; 18 had had genetic testing and 13 of these had been shown to have FAP. Moreover, 36% fulfilled criteria for a psychiatric disorder; among those over age 15, the percentage was 43%, significantly above both general population norms for Norway (15%). Worry about parental health or possible death of a parent was high among the FAP cohort; there was a trend for children of maternal FAP carriers to have more psychiatric problems. Interestingly, personal FAP status and age at testing did not seem to be associated with the level of psychiatric problems. An Australian study of individuals 14–26 years of age tested for FAP also reported potential harms of testing, including considerable worry for the pain experienced by parents regarding their children's risks, as well as documenting potential benefits of testing which included relief when testing negative, strengthening of bonds with other affected family members when testing positive, and increased clarity about cancer risk [51].

There are a number of studies which provide similar general findings about psychosocial outcomes for FAP-affected young adults (typically in their 40s), though there are also some differences reported in terms of the extent of distress and the impact of surgery. In general, as with studies of hereditary breast ovarian cancer (HBOC) and other adult-onset hereditary cancer syndromes [52], individuals affected with FAP and those at high risk for FAP in most studies do not typically show distress in clinical ranges or evidence of major psychiatric illness in response to the stressors of living with FAP [53–55]. However, FAP-specific measures of quality of life suggest considerable compromise for postsurgery patients, especially those who are single [56]. These findings suggest the importance of longer-term follow-up post-FAP surgery to help young patients to cope with their continuing physical and psychological concerns.

Given the potential psychosocial impact of FAP on young people, recommendations for patients with FAP include that psychosocial counseling be offered to FAP patients both before surgery and afterward to inform them about the social and practical consequences of surgery and to help with postsurgical adjustment. Other recommendations include education of healthcare providers about FAP, FAP educational programs, and meetings with similarly affected peers to reduce isolation [55, 57]. Recognition of the rights of autonomy of teenagers and having time after counseling and before genetic testing to consider the impact of the test result are also recommended [58].

15.6.2 Genetic Testing for FAP in Childhood

Early research showed 85% uptake by at-risk adults and 96% by at-risk children presenting for genetic testing for FAP [59]. Another early study suggested ambivalence on the part of the 10% of subjects who signed up for genetic counseling and testing but later withdrew [60]. Codori [49] reported that 35% of parents who offered genetic testing for at-risk children declined to have their children tested. More recently, a Dutch group interviewed 8 parents of 13 children tested for FAP

under the age of 10 (which is younger than usually offered to families) [61]. Parents reported that they requested testing to provide some certainty regarding their child's future, to prepare the child for future screening, and to align with timing of testing for older siblings. Most parents reported preferring to share the test result with their child themselves rather than relying on a professional. Parents in this study did not report any significant changes in their child's psychosocial well-being after testing and reported that they did not regret having their child tested [61].

15.6.3 Screening in Children with FAP

A 2008 report from the Netherlands found that 20% of individuals 16 years or older at risk for FAP and 25% of those who already had surgery and had a retained rectum were not compliant with screening advice [62]. Some had no screening at all, and others had one or two endoscopies, but did not follow advice for annual screens. Factors associated with noncompliance were the unpleasantness of the exam and the lack of sedatives for the screening test, resulting in more procedural and post-procedural pain [63]. Compliant individuals had stronger beliefs about the efficacy of screening and higher self-efficacy about being able to accomplish screening regularly. Recommendations were offered to increase the use of sedatives before and after screening for FAP [62]. Clearly, young people at risk for FAP require careful education so that they can understand the benefits of screening and can feel empowered to make informed decisions about utilizing screens to prevent colorectal cancer and reduce their risk of dying from their inherited cancer predisposition. As Chapman and Burn [64] put it, they must have "a belief that it is possible to remain healthy while having an increased risk." Hyer and colleagues [47] provide useful recommendations to improve screening compliance in young people with FAP, including enrolling children and young people in a polyposis registry, ensuring that clinicians develop trust and empathy with young people, and providing psychosocial support to families [47].

15.6.4 Reproductive Decision-Making

A very small sample ($n = 20$) of adults with FAP were surveyed about their attitudes and potential use of prenatal genetic testing to prevent the birth of a child with FAP [65]. In this sample, 35% felt that having FAP had affected their decisions to have children. Furthermore, 100% of those planning future pregnancies were willing to consider prenatal diagnosis (PND), with a strong preference for pre-implantation genetic diagnosis (PGD) to avoid the possibility of having to terminate a pregnancy. A preference to avoid the ethical issues related to pregnancy termination has been a consistent finding in studies of prenatal genetic technologies for other hereditary diseases [66]. A critical issue is, however, the lack of information about

reproductive options among FAP family members of reproductive age, with 84% in one study [67] having no knowledge of these available technologies prior to the survey.

15.7 Retinoblastoma (Rb)

Retinoblastoma, an aggressive eye cancer typically diagnosed in the first 1–2 years of life, was the earliest pediatric hereditary cancer syndrome to be identified; investigation of hereditary retinoblastoma is said to have "transformed the thinking about cancer" [68]. Hereditary retinoblastoma, due to dominant mutations in the *Rb1* gene [69], is typically diagnosed earlier than sporadic retinoblastoma [68] and is more likely to affect both eyes. The survival rate of retinoblastoma is high in Western countries. A study in the USA reported 5-year survival rates of 96.3% for children with unilateral retinoblastoma and 92.5% for children with bilateral retinoblastoma [70]. However, hereditary retinoblastoma conveys significantly higher risk for second or even third malignancies throughout life, necessitating lifelong surveillance [71–73]. Delays in diagnosis can impact mortality [74], resulting in potential emotional ramifications for parents in cases where diagnosis is not rapid.

A tenet of the approach to treatment of retinoblastoma is that saving lives is more critical than saving vision [75]. For children found to have large eye tumors, enucleation or removal of the eye, typically replaced by a prosthetic implant, is curative but carries with it significant risk for later emotional and educational difficulties [68]. For smaller tumors, chemotherapy with focal laser treatment and cryotherapy or sometimes radiotherapy may be utilized without loss of vision in that eye, but this may reduce the child's likelihood of survival. In families where a parent carries an *Rb1* mutation, fetuses can be tested to determine whether they are mutation carriers, and, if so, premature delivery around 36 weeks of gestation and immediate treatment of any small tumors which are found is recommended [68].

15.7.1 Psychosocial Outcomes of Retinoblastoma in Childhood

The emotional consequences of retinoblastoma are heavily influenced by the early age of onset (affecting the early parent-child relationship), by the cosmetic and functional outcomes, (affecting social and educational success), and by the presence or absence of second malignancies (affecting morbidity and mortality). Qualitatively, parents have reported feeling guilty or responsible for their child's disease and that the diagnosis has impacted relationships with family and friends [76]. Parents report a desire for their child with retinoblastoma to lead a "normal life" but experience frequent worry about their child's future cancer risk and potential vision loss [76]. Families also report a need for more psychosocial support and support to communicate with children and peers [76].

There is a small body of quantitative data examining the psychosocial outcomes of retinoblastoma patients. Many of the outcomes are positive, despite retinoblastoma significantly impacting many aspects of the child's life. A study of 156 Dutch retinoblastoma survivors, ages 8–35, found that, compared to the Dutch general population, survivors of retinoblastoma did not differ in rates of employment or marital status. Only 4% were unable to work because of their disease, although 26% believed having retinoblastoma had influenced their choice of career. Satisfaction with friends was high, and the levels of interpersonal interaction were similar to the general population. However, 55% felt restricted in their daily lives to some extent by the retinoblastoma. Worry about future cancers was reported by 6% of the younger survivors and by 15% of the adult survivors. Moreover, 18% worried about further loss of vision [77]. Anxiety and negative self-esteem were high, and 28% reported hesitation about social interaction related to fear of being rejected. In addition, 48% of the survivors reported having been bullied at some point compared to a societal norm of 18% [77].

In an English study [78], parents of about 80% of the retinoblastoma survivors reported their child had been teased by peers about their facial appearance. Bullying in childhood was a significant predictor of reduced emotional, physical, and social functioning, as measured by the SF-36 among adult retinoblastoma survivors [79]. The English study, however, also found good school attendance, good performance on verbal IQ measures, and little need for special schooling among retinoblastoma survivors. Those who were blind or partially sighted had, as might have been expected, lower performance IQ scores. On the positive side, all the survivors took part in physical activities and after-school activities, and few reported problems in forming friendships. Mothers seemed to report greater behavioral problems than were reported by the children themselves [80]. Greater behavioral problems were reported among survivors who had undergone intensive treatment (who tended to be those with hereditary retinoblastoma) or who were from single-parent or divorced families.

15.7.2 Genetic Testing for Heritable Retinoblastoma

Genetic counseling and testing for heritable retinoblastoma is critical to identify patients' and family members' future cancer risk [76]. In many countries, genetic counseling and testing is considered standard care for all children with retinoblastoma [76]. Hill and colleagues [76] conducted 3 focus groups with 13 parents of children with retinoblastoma and 2 retinoblastoma survivors, exploring their knowledge of retinoblastoma genetics and experiences with genetic testing. Participants generally understood that retinoblastoma was a genetic disease but revealed misunderstandings of key genetic concepts, for example, misunderstanding the difference between the terms "genetic," "inherited," and "heritable" [76]. Despite this, participants reported that genetic testing benefited families, particularly by enabling early identification of risk to the patient and their family members. Participants shared

that accessing easy-to-understand genetic information was difficult and that genetic information was received at a very stressful time (during cancer diagnosis) [76].

15.7.3 Screening in Children with Retinoblastoma

Dedicated screening of newborns and children at risk of retinoblastoma is critical for early detection of tumors, with the goal of curing the disease without total loss of vision [81, 82]. While many germline cases of retinoblastoma arise from de novo mutations, genetic testing of siblings and first cousins can enable those found not to be mutation carriers be spared the need for recurrent screening [83]. There is little data available on the psychosocial impact of retinoblastoma screening during childhood, although it is clear that parental compliance with screening recommendations is influenced by multiple factors, including the family's demographic and socioeconomic characteristics, cultural beliefs, concerns about discrimination, and barriers to accessing medical care [81]. One recent study reported that while most parents of children with retinoblastoma understand the disease is heritable, they do not always fully understand the need for screening for their other children [81].

15.7.4 Reproductive Decision-Making

Concern about having children (or further children) with retinoblastoma leads many affected individuals or their parents to alter their reproductive decision-making [76, 84, 85]. The majority of parents of children with retinoblastoma in one 2010 study ($n = 81$) opted not to have more children, 12% were sterilized, and 20% chose to utilize chorionic villi sampling for prenatal diagnosis which led, in one case, to termination of pregnancy [86]. Perceived risk was the major factor predicting reproductive behavior. Perceived risk was notably often markedly different from the objective risk, which led the authors to suggest that continuing access to genetic counseling should be available even after genetic diagnosis and treatment for individuals with retinoblastoma [86].

15.8 Von Hippel-Lindau Syndrome

Von Hippel-Lindau (VHL) syndrome is characterized by a number of benign and malignant tumors. These include pheochromocytomas, hemangioblastomas of the retina, cerebellum, spinal cord, renal clear cell carcinomas, and pancreatic cysts. Age of onset is widely variable, ranging from early childhood to late adulthood [87]. Screening is advised to begin at age 5 with annual ophthalmologic examinations and periodic screening of multiple organs.

15.8.1 Psychosocial Outcomes of Retinoblastoma in Childhood

Little data exists on the psychosocial impact of VHL, and most of what does exists focuses on adults with VHL. A Dutch study of 123 family members, ages 16–83, from 38 families affected by VHL included 68 carriers of a *VHL* mutation, 39 proven non-carriers, and 16 individuals at 50% risk [20]. Cancer worry was high, with 38% of carriers describing frequent concern about developing cancer or an additional cancer and 46% worried about having to undergo surgery in the future due to VHL. Further, 39% of the participants had scores indicating at least moderate distress on the Impact of Event Scale [88], a measure of cancer-related distress; 13% had distress scores in the severe range. Worry about family members developing tumors was also common. As with other cancer syndromes, perceived risk and the experience of a death of a close family member due to the hereditary cancer syndrome especially during adolescence were major factors predicting distress of family members [20]. In general, quality of life scores were similar to Dutch population norms, except on the "general health" subscale.

A more recent Australian study reported similar themes for adults with VHL [89]. With regard to children, the study reported that parents can experience a sense of transmission guilt for passing VHL to their children and that it was difficult to meet the needs of their affected and unaffected children [89]. Both the Dutch and Australian study authors recommended periodic psychosocial screening to identify family members most in need of referral for psychological support [89, 90].

15.8.2 Genetic Testing for VHL in Childhood

Genetic testing is offered to children at risk of VHL because some VHL patients develop tumors in childhood [91]. While parents typically would prefer to have their child tested before age 10, some research suggests that testing can evoke feelings such as anxiety, denial, and guilt in affected family members, and some parents express concern about making genetic testing decisions on behalf of their child [91].

15.8.3 Screening in Children with VHL

It is accepted that VHL screening programs can improve outcomes in individuals with VHL, usually commencing in childhood [91]. However, VHL families describe the "anxiety-provoking" burden of screening, leading to some individuals to not adhere to their recommended screening regimen [91, 92].

15.8.4 Reproductive Decision-Making

Pre-implantation genetic diagnosis (PGD) for VHL is available as a means of reducing the risk of having a child who is subject to the disease risks of VHL. Moreover, 33% of members of families affected by VHL surveyed in a Dutch study expressed positive attitudes toward the use of PGD, though many of these individuals were not currently intending to have children [44]. Among those who were planning (more) children, interest was higher, though none of the participants had actually utilized PGD. Partners of VHL patients had similar levels of interest in PGD. No psychosocial factors significantly predicted interest in PGD.

15.9 Multiple Endocrine Neoplasia Type 2 (MEN2)

MEN2A is a cancer syndrome caused by mutations in the *RET* gene [93]. Since 1993, direct mutation testing has been available, making it possible to determine which family members require the complex thyroid and other screenings necessary to reduce morbidity and mortality in this group of high-risk individuals and which do not. Medullary thyroid cancer (MTC) develops in nearly all mutation carriers who do not undergo surgical removal of the thyroid before the age of 35 [94]. MTC grows slowly but has few clinical signs and thus, without screening, may have metastasized before it is detected, which can have fatal consequences. The surgery itself is relatively straightforward, and lifelong thyroid hormone replacement reverses the consequences which would otherwise occur in the absence of thyroid. Because of the additional high risk for pheochromocytomas (adrenal tumors) in MEN2A and MEN2B, biochemical screening of adrenal function is also required, and surgical removal of one or both adrenal glands may be necessary.

In some subtypes of MEN2, thyroid removal is recommended as early as 12 months [95], but the average age at diagnosis is 20 years, well below age 35, the mean age for diagnosis of nonhereditary MTC. The age at which prophylactic thyroidectomy is recommended may be different depending on the location of the *RET* mutation, though these guidelines continue to be modified [94, 96]. Biochemical screening is advised to begin in children with MEN2B at age 6 months and ages 3–5 years for those with MEN2A [94].

15.9.1 Psychosocial Outcomes of MEN2 in Childhood

While there are clear medical advantages to identifying *RET* mutation carriers, it is also understandable that there may be significant psychological ramifications. The psychosocial literature remains limited about the impact of MEN2 on children. Early findings suggest a need for careful psychological and genetic counseling prior

to testing [97]. Some parents report feelings of guilt for passing MEN2 to their children and disagree about how to manage mutation-positive children [98, 99]. One recent study reported that parents who passed on a *RET* mutation to their child had higher "anxious preoccupation" and "discouragement" scores and lower cognitive, emotional, and physical functioning scores than parents of non-carrier children and patients without children [98]. Mutation-negative children sometimes resent the focus on the mutation-positive child but also can feel guilty to be spared ongoing screening. Children can also feel reluctant to discuss MEN2 with friends. Giarelli [100, 101] focused on the self-surveillance which is required to monitor physical symptoms, medical visits, laboratory findings, taking of medicines, etc. and on the emotions which such constant reminder of illness and potential exacerbation of symptoms engenders. There are also positive effects for some of taking control over complex, hereditary medical concerns. The factors predicting an individual's ability to balance these emotions await further study.

15.10 Summary of Findings Regarding Specific Syndromes

Clearly, the identification of high hereditary cancer risks can increase anxiety among both parents and children in an affected family. The need for multiple forms of lifetime surveillance increases the medicalization of children in hereditary cancer families; genetic testing can identify those who can be spared repeated screenings over many years, creating inequities which may be difficult for some children to understand and accept and for parents to manage [11]. Identity issues also affect these children, leaving them feeling isolated from peers and carrying an added layer of concern about the challenges of dating, marriage, and childbearing with so many interlocking medical issues [11]. Research is sorely needed about the particular psychosocial stressors and predictors of distress in the different syndromes but will likely have to involve creation of international consortia, as most are rare syndromes. Understanding these issues can help mental health professionals develop models of effective genetic and psychological counseling services for children and parents living with hereditary cancer syndromes.

15.11 New and Future Concerns

15.11.1 Direct to Consumer Genetic Testing

Direct to consumer genetic testing (DTC) offers genetic testing to consumers, without the involvement of health professionals [102]. Many companies offer analyses which can cover both pediatric-onset and adult-onset conditions with no prior genetic counseling, though some companies offer consumers access to genetics

counselors. Interest in genetic testing of children and adolescents is high among parents and young people [11, 103, 104]. Several studies show that the majority of parents report an interest in using DTC, with most parents reporting a willingness to submit genetic material of their children even though many do not understand the risks and benefits of using DTC testing [105, 106]. A study of 219 parents of minor children who were offered multiplex genetic testing for eight serious common conditions through their health management organization were queried about their interest in having their children tested [107]. Parents gave greater weight to the expectation that testing would offer reassurance about conditions their children were not at high risk for, leading many parents to desire testing of their children. Data from this study gave the researchers reason to be concerned that many parents might feel regret following testing and might find it difficult to cope with disappointment in learning about genetic risks faced by their children. While there has been some controversy within the genetics community about whether research should be conducted involving children in DTC genetic testing [102, 108–110], the predominant view of pediatrics and genetics professionals is that DTC testing of children should discouraged until a time when companies can provide clear evidence of the quality and accuracy of their tests and adequate pre- and post-genetic counseling to families [102]. The American Society of Human Genetics further recommends that DTC testing for adult-onset genetic conditions is not undertaken in children [102].

15.12 Whole-Genome Sequencing and Other Tests for Multiple Genes

Previously, most genetic testing for cancer risk involved the search for mutations in one or a handful of genes related to the etiology of a particular condition. However, recent reports using genome-scale germline sequencing of children with cancer suggest that at least 10% of child cancer patients carry a germline mutation in a known cancer predisposition gene [14–16]. This discovery, coupled with the decreasing cost, has resulted in increasing numbers of children being offered testing across a broad panel of genes or testing of the whole genome or exome. This testing is increasingly being offered to families at cancer diagnosis or at relapse in the context of precision medicine programs aiming to use genetic information from the child's tumor and germline to make personalized treatment recommendations for the child [111, 112]. This new approach will significantly alter the future clinical management of children with cancer. Early identification of an underlying cancer predisposition syndrome in a child with cancer could alter management of the child's current disease and guide longer-term screening and other risk reduction recommendations for children and their family members [113]. Despite this exciting potential, there is little data on the short- and long-term psychosocial impact on children and their families [103, 114]. Early studies suggest that young patients and parents may hold

high expectations for germline sequencing, for example, believing that the testing, or the precision medicine approach, will significantly increase the child's chance of cure [103, 115]. Given the complexity of whole-genome sequencing and precision medicine, it is not surprising that there is also early evidence that young patients and parents misunderstand key concepts, may feel overwhelmed by large amount of information provided, and may feel distressed by the speed with which decisions about testing need to be made [103]. Further research is needed to better understand families' experiences of and attitudes toward receiving genetic information about cancer risk via whole-genome sequencing and precision medicine programs.

15.13 Conclusions

Understanding more about the psychological impact of hereditary cancer, genetic testing, whole-genome sequencing, and long-term screening on children from families affected by syndromes which predispose them to cancer at early ages is essential if we are to be able to determine the impact of advancing technologies and to provide psychosocial support to these vulnerable children and their parents. We need direct assessment from the children themselves, true patient-reported outcomes, to understand which are the moments of greatest fear and which groups of children require the most support [2, 103]. These observations will help us to understand and build on the factors predicting resilient integration of knowledge of hereditary cancer risk into young people's lives. We need to learn what types of genetic information are most helpful to young people, how it can be most effectively delivered, and how we can help them to feel less alone and empowered to manage their health. We also need to understand how to help parents and children talk to each other about hereditary cancer and about lifelong screening and other options which can potentially save lives [11]. Understanding how to manage the transition to adult providers for young people in hereditary cancer families and how to help them communicate about hereditary cancer with significant others could reduce their cancer-related distress and support positive health management [103].

Study of the psychosocial impact of hereditary pediatric cancers is complicated by lack of standard methodology. The deeper we look, the more qualitative the responses, the more likely we are to find distress and difficulty. This is not dissimilar to the findings about the impact of adult-onset hereditary cancer syndromes. Living with such risks typically does not bring on lifelong psychiatric conditions, it seems, but they do add challenging layers of anxiety, self-doubt, and fear for the future which professionals should attend to in the care of hereditary cancer families. As with pediatric cancer generally, it is also possible that there are some positive outcomes of living with hereditary risk in childhood, such as possibly early maturity, increased family bonds, greater self-confidence about handling future crises, and a sense of life seeming more precious which have not yet been fully studied in the pediatric hereditary cancer population [2].

The data also suggest that members of families affected by pediatric hereditary cancer syndromes need lifelong access to genetic counseling, even long into survivorship [102, 116]. For young people, there are many points at which genetic counseling could be informative and supportive as they mature into adults. It may be helpful to differentiate between "knowing for the sake of knowing" and "knowing for the sake of action" [107]. Parents need help in determining how and when to inform their young offspring [117, 118]. Young adults need help making decisions about living with hereditary cancer risk, sometimes before they are ready for genetic testing. Young adults making decisions about the use of reproductive technologies to avoid the birth of an affected child may well need to discuss that option with genetic counselors and mental health providers, in terms of both the costs and physical risks and the implications for their own identity and values which such a decision may imply. Similarly, discussion of the psychosocial and practical issues regarding what is often lifelong surveillance/screening and decision-making about risk-reducing surgical options are important to reach optimal outcomes. It is important that young people have early consultation options and adequate time to come to the right decision about when to seek testing or initiate screening or undergo surgery. Young people in hereditary cancer families appear to worry about the cancer risks of their offspring, but they also worry about the cancer risks to their parents, siblings, and other relatives and may need support around these anxieties [11]. Psychosocial as well as genetic counseling support should be easily available and provided by individuals knowledgeable about hereditary cancer syndromes so that these young people and their parents will feel understood and will not have to educate their providers [11]. This will necessitate considerable training of mental health professionals who are not typically aware of the implications of such inherited risk [119].

It is clear that international consortia will be needed to study the psychosocial issues relevant to patients in hereditary cancer families [120]. While many of the concerns are similar across the pediatric hereditary cancer syndromes, there are individual issues of importance for patients and family members related to the particular cancers which occur, the age of onset, and the types of screening or risk-reducing surgeries which are recommended. To develop cohorts of a sufficient size to study the resulting psychological impacts, the timing of peak concerns, problems in family communication, and other issues, cooperation across national borders will be essential. Developing cancer registries which allow us to easily make contact with patients whose families meet criteria for hereditary cancer syndromes would rapidly advance work on the critical medical and psychosocial questions. Future research will require considerable creativity in funding and careful consideration of methodological comparability of measures and ethical issues about privacy and research access. New technologies of the future will offer new options to families who are affected by hereditary cancer, but they will also raise new and challenging questions about identity and access which we need to understand in order to better serve our patients.

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Chapter 16
Recognition of Cancer Predisposition Syndromes

Lara Reichman and Catherine Goudie

Abstract This chapter aims to explore the multitude of challenges that limit the clinician's ability to rapidly identify cancer predisposition syndromes in children with cancer. The current clinical approaches as well as novel strategies for CPS screening and detection will also be discussed. In particular, the integration of comprehensive germline sequencing and the development of eHealth technologies in pediatric oncology practice will be presented in this chapter.

Keywords eHealth tools · Risk assessment · MIPOGG · Artificial intelligence · Testing algorithms

As this book highlights, pediatric cancer predisposition syndromes (CPSs) are complex and variable conditions. The broad range of phenotypes and sheer number of CPSs complicate their timely diagnosis. In this chapter, we will first explore the current standard-of-care approach for CPS detection, physician-based recognition, and then elaborate on newer strategies, including a universal testing approach as well as the implementation of scoring systems and eHealth tools for CPS risk assessment.

L. Reichman
Department of Medical Genetics, McGill University Health Centre, Montreal, Canada

C. Goudie (✉)
Division of Hematology-Oncology, Department of Pediatrics, McGill University Health Centre, Montreal, Canada
e-mail: catherine.goudie@mcgill.ca

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16.1 Physician-Based Recognition

It is estimated that 8–10% of children diagnosed with cancer have an underlying CPS [1, 2]. Pediatric oncologists have a unique opportunity to screen for CPSs at the time of primary cancer diagnosis in their patients. For a clinician, the first step in diagnosing a CPS in a child is recognizing the possibility of an underlying syndrome; traditionally, detecting a genetic association to cancer has relied on clinicians' knowledge and ability to identify relevant patterns of physical manifestations or family history. This process is met with multiple challenges, limiting a clinician's capacity to quickly assess for a CPS in a child. Rapid technological advances in genetic sequencing continually lead to new discoveries in cancer genetics, making it difficult for physicians to keep abreast of the expanding evidence base on heritable cancers. The majority of physicians, including oncologists, are also not specifically trained in cancer genetics and therefore develop their knowledge on the subject through past experience. With over 125 types of CPSs currently associated with pediatric cancer development, many physicians may have limited previous encounters with patients having one of these CPS diagnoses. Additionally, many CPSs are associated with a widening spectrum of tumors, making their diagnosis even more complex for clinicians.

The majority of children with CPSs are diagnosed with cancer prior to the knowledge of their underlying genetic diagnosis. In part, this sequence of diagnoses is linked to parents having rarely consulted for other less striking phenotypic features linked to a CPS. For example, a hamartoma in a patient with PTEN hamartoma tumor syndrome may have been missed if not considered problematic by families. Café-au-lait spots present no physical impact on daily life and may not be readily visible to parents. In families in which a CPS is inherited, other physical features, such as frontal bossing or supernumerary teeth, may be assumed to be familial traits, without consideration for a possible underlying CPS, especially if cancer diagnoses have been few or nonexistent.

Even after a child is diagnosed with cancer, there is high variability in their likelihood of being recognized with a CPS depending on where and by whom they are treated. In urban centers, the initial physician-based assessment for a CPS is usually part of the review of past medical history and family history by a pediatric oncologist. Certain patients may be seen by a surgical team if the tumor solely requires a surgical resection without systemic treatments. This may lead to divergent approaches to evaluate the likelihood of a CPS in a young patient. As one might imagine, questions surrounding a CPS are intertwined with crucial questions assessing for possible comorbidities and risks for toxicity and complications from upcoming treatments; noticing the pattern of a CPS at the same time can therefore be extremely difficult.

The frequency of genetic referrals and testing practices can also vary drastically based on numerous factors including cancer presentation, clinicians' individual knowledge and practice, as well as institutional guidelines. The geographic,

socioeconomic, demographic, and cultural background surrounding a healthcare center and its resources can also limit the possibilities of genetic evaluation.

For example, a child presenting a Wilms tumor may be automatically sent for genetic testing in certain tertiary/quaternary care hospitals with easy access to a genetics service and clinical genetic sequencing. In other hospitals, the possibility of an underlying CPS may not even be raised. Alternatively, some clinicians would like to offer genetic testing to a patient presenting with a Wilms tumor, but cannot easily access a genetics service or testing.

The main limitation in physician-based CPS recognition is the inequality in a patient's likelihood of being identified with a CPS. This can lead to missed opportunities for cancer surveillance and for early detection of additional malignancies in the patient and their family members.

To aid the physician-guided approach, different criteria, questionnaires, and guidelines have been developed. For example, in 2017, Ripperger et al. published recommendations for CPS recognition by the Cancer Predisposition Working Group of the Society for Pediatric Oncology and Hematology [3]. These recommendations for "when to consider a CPS" are concise and include crucial "red flags" for CPS identification. Similarly, in 2017, Jongmans et al. published an easy-to-use selection tool based on a questionnaire including specific features in the cancer presentation and personal and family history that would raise suspicion for a CPS [4]. Many other educational publications exist that describe the known associations between various tumor types and CPSs [5–10]. Another scoring system for CPS recognition is the "traffic light" classification approach developed by the Cancer Genetics Unit in the Royal Marsden Hospital, NHS Foundation Trust. This approach classifies certain cancer diagnoses and associated features into a red, amber, and green light category, depending on the genetic evaluation recommendation [11]. These are examples of publications that link certain cancers to one or many CPS types (i.e., they start from a specific tumor type and lead to the recognition of a CPS). While these manuscripts are extremely helpful at the time of publication, they can become outdated quite quickly as new discoveries are made.

Alternatively, many publications present scoring systems and questionnaires for certain CPS types (i.e., they start from a specific CPS type and present criteria for genetic testing or clinical diagnosis). For some CPSs, identifying a sufficient number of distinguishing features can lead to a clinical diagnosis, such as for neurofibromatosis type 1 (NF1) [12]. Features of NF1 were initially laid out by the NIH in 1988; these include six or more café-au-lait macules over 5 mm, two or more neurofibromas or one plexiform neurofibroma, freckling in the axillary or inguinal region, an optic glioma, two or more Lisch nodules, a distinctive osseous lesion such as sphenoid dysplasia or tibial pseudarthrosis, and a first-degree relative with NF1. While some of these individual characteristics are not pathognomonic on their own, when enough of them are paired together, they are highly sensitive and specific for an NF1 diagnosis. In the case of NF1, options for a clinical diagnosis are especially useful given that sequencing of only genomic DNA can miss a significant proportion of the causative variants related to splicing. However, as described, many

of these criteria are dermatologic features and may require time or examination devoted to evaluation of these NF1 characteristics.

Other criteria to aid physician recognition also use a specific CPS as the starting point for assessment. The Chompret criteria is one such example used to evaluate the likelihood of Li-Fraumeni syndrome (LFS) [13]. LFS is a CPS that presents solely with cancer diagnoses and no other physical manifestations. Mai et al. estimate that approximately 30% of those meeting Chompret criteria will have a germline pathogenic variant in *TP53* [14]. Using these criteria requires recognizing that the presenting cancer type may be indicative of LFS. This recognition is also dependent on a sufficiently penetrant CPS whose impact can be assessed in the family; if family members with the CPS are presently unaffected or the specifics of their diagnoses are not known to the rest of the family, it can complicate the recognition of characteristic features. Similarly, using these criteria assumes that the *TP53* variant is inherited and does not account for up to 20% of people with LFS who have de novo variants and therefore lack a striking family history [15]. In 2014, Wimmer et al. compiled a point system for constitutional mismatch repair deficiency (CMMRD), weighing characteristics of the CPS differently to guide when to offer a genetic test to a patient [16]. They suggest that patients with a score of 3 or higher are much more likely to have a diagnosis of CMMRD, and testing in this case is for a confirmation of the suspected diagnosis.

In both the Chompret criteria and Wimmer et al.'s point system, some knowledge of particular aspects of the family history is required for a complete assessment. Family histories are often inconsistently documented by physicians and are absent altogether in approximately half of patients [17]. Parents do not always disclose cancer diagnoses in other family members (or may not even be aware of these events). With worldwide immigration, many families do not stay in touch with other members. Perceived taboos of sharing news of cancer diagnoses among certain families may also limit the ability to obtain an accurate family history [18, 19]. An additional challenge in pediatrics is that young children have young parents and siblings who may not yet have developed any cancers or CPS-related phenotype, making it even more difficult to interpret seemingly "negative" family histories of cancer. As a result, rather than depending on potentially unreliable or inconsistent assessments of family history, researchers and clinicians keen on identifying the maximum number of children with CPSs have advocated for "universal" genetic testing [20].

16.2 Universal Genetic Testing

In contrast to the physician-based recognition approach, certain oncology institutions are collaborating together to implement a "universal genetic testing" strategy, either on a research and/or clinical basis. Universal genetic testing implies "testing everyone" for variants in a broad number of cancer-related genes with increasingly sophisticated and accessible next-generation sequencing (NGS) techniques. NGS

and large cancer gene panels have revolutionized the options available for mass genetic testing while decreasing the time and cost needed for sequencing, allowing testing of all children with cancer, without any additional selection criteria. This universal testing approach typically involves the sequencing of the tumor DNA in addition to the germline DNA.

In 2015, St. Jude Children's Research Hospital began the Genomes for Kids (G4K) clinical trial [21]. This study has many aims, one of which is to increase identification and better understand the prevalence and spectrum of CPSs. By having clinical information available about participants as well, they are able to correlate the genetic findings (from a large cancer gene panel) with a possible clinical presentation. The onus to recognize the possibility of a CPS, let alone a specific one, therefore no longer falls on the treating physician—their responsibility shifts to an introduction to, or discussion of, this research option. In a universal testing approach, the provider's role changes from recognizing characteristic CPS patterns to providing an opportunity for comprehensive genetic sequencing. Many other institutions and collaborations worldwide have ongoing pediatric cancer sequencing programs similar to the G4K [22–26]. This approach also alters the possible range of results as universal genetic testing typically implies large multigene panels. These panels increase the likelihood of dealing with challenging variants of uncertain significance (VUSs), genetic changes for which the clinical impact cannot be determined at present. VUSs are a well-established source of confusion for providers as well as patients; a recent study of adult patients undergoing their own oncology-related genetic testing revealed that even when patients grasp that the result is uncertain, they still assign a level of clinical significance to their VUS [27]. Interpreting significance in the pediatric context has the potential to be even more damaging, causing parents to possibly assign a genetic risk for cancer to their other children or family members. While these VUSs are inevitable and need to be studied to eventually understand their potential role in tumor development and evolution, these uncertain findings can be confusing and add to the stress for a family dealing with a young patient's recent cancer diagnosis [28]. Other complex findings can include variants in adult-onset cancer genes and carrier statuses. Indeed, universal genetic testing allows for a broader scope, including the analysis of genes clearly related to CPSs but is perhaps adult onset (e.g., *BRCA1*). Identifying a pathogenic variant in a gene like *BRCA1* may be important, even in a child, for consideration of potential treatment opportunities (e.g., PARP inhibitors [29]), but it requires balancing this possible finding against less clear results. In other cases, variable inheritance patterns may complicate the exclusion of adult-onset CPSs from pediatric testing. Certain pediatric CPSs are inherited in an autosomal recessive manner: Fanconi anemia or constitutional mismatch repair deficiency, for example. However, testing for such syndromes is complicated by heterozygous pathogenic variants in the related genes causing adult-onset CPSs: *BRCA2* or *PALB2*-related hereditary breast and ovarian cancer, or Lynch syndrome (related to heterozygous variants in *MLH1*, *MSH2*, *MSH6*, or *PMS2*). The complexity of these different inheritance patterns and ages of onset involving the same genes can add another dimension of complexity when considering clinical multigene panel testing. Analyzing these genes in a

research context allows for decision-making around disclosing to patients and families only the results that seem to explain their phenotype.

The value of the universal testing approach emerges more from the pathogenic/likely pathogenic variants identified in those patients *not* meeting clinical criteria for a given CPS. By providing universal genetic testing, the maximum number of patients with CPSs is identified, and this is especially worthwhile for patients who are unexpectedly found to have a CPS. This broad approach to genetic testing offers an opportunity to identify CPSs in families in which there may not have been an adult-onset diagnosis yet, thereby allowing access to earlier screening and preventive measures for those family members with the CPS. Large multigene panels also allow the identification of associations that may not previously have been known or considered, such as a variant in a gene not typically related to the presenting tumor type. The question remains from a clinical perspective, how to integrate this new information into meaningful surveillance, and prevention for patients and their families. While costs of sequencing are continuing to decrease, making universal testing seem more financially appealing, there remain significant requirements for bioinformatics with expertise in oncology and appropriate infrastructure to interpret and transmit these genetic findings to physicians and patients. The importance of an appropriate patient and parent consent process performed by a health professional with expertise in genetics cannot be underestimated.

A harmonization of the yield from universal genetic testing with the increased precision from a physician-based approach would help fine-tune CPS detection in children with cancer. eHealth tools, such as apps, are one way of providing more structure around the identification of CPSs while eliminating the physician-dependent knowledge bias.

16.3 eHealth Tools

eHealth is an opportunity to streamline CPS risk assessment. There are different types of publicly available eHealth programs. In adults, numerous Internet-based risk calculators exist for specific CPS diagnoses (Table 16.1).

These programs are mostly dependent on having a suspicion for a particular CPS and therefore still rely on some physician knowledge to guide their use/initiation. However, they are more interactive than static risk assessment models. The PTEN calculator, for example, provides the likelihood of a pathogenic variant in *PTEN* as a percentage based on responses/total score to the questions presented. Likewise, the PREMM 5 model adjusts the likelihood of a pathogenic variant in *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM* based on how the questions are answered. Again, the major limitation stems from the risk assessment originating from a specific CPS type. Gastrointestinal carcinomas can be seen in both PTEN hamartoma tumor syndrome and Lynch syndrome. If a patient presented with this cancer, a clinician would need to run both the PTEN calculator and the PREMM 5 model to assess for both these CPSs; this does not begin to factor in the numerous other CPSs

Table 16.1 Examples of web-based risk calculators for various cancer predisposition syndromes

Model name	Cancer predisposition syndrome	Website/publication
PREMM 5 Model	Lynch syndrome	https://premm.dfci.harvard.edu/
MMRPro	Lynch syndrome	https://projects.iq.harvard.edu/bayesmendel/mmrpro
PTEN risk calculator	PTEN hamartoma tumor syndrome	https://www.lerner.ccf.org/gmi/ccscore/
The Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA)	Hereditary breast and ovarian cancer	https://ccge.medschl.cam.ac.uk/boadicea/boadicea-web-application/
BRCAPro	Hereditary breast and ovarian cancer	https://projects.iq.harvard.edu/bayesmendel/brcapro
Tyrer-Cuzick	Hereditary breast and ovarian cancer	https://ibis.ikonopedia.com/
MEN1 Nomogram	Multiple endocrine neoplasia type 1	DOI: https://doi.org/10.1530/EJE-12-0210 (paper based)

in which gastrointestinal carcinomas can be seen. The MEN1 nomogram [30], while in a static paper format, provides a percentage for the likelihood of a *MEN1* pathogenic variant based on the responses and therefore has a similar ability to provide a range of risk depending on the answers, like the PTEN calculator and PREMM 5 model. Nevertheless, a patient's risk for all related CPSs would not be assessed using this current structure of questions beginning with the CPS as opposed to the tumor type.

One decision support tool that has recently been launched is the McGill Interactive Pediatric OncoGenetic Guidelines (MIPOGG) [31]. MIPOGG is an evidence-based decision support tool that incorporates all currently known CPS types for all pediatric cancers. It consists of 140 tumor-specific decisional algorithms that generate a recommendation for "referral" or "no referral" for genetic evaluation based on the likelihood of a CPS. This tool is designed for physicians who encounter children newly diagnosed with cancer. MIPOGG uses clinical, family history, and tumor-specific features to streamline genetic referrals and testing. Moreover, the starting point of a tumor type, as opposed to a CPS type, makes it much quicker to use and more complete for physicians than having to consider all the CPSs which might be linked to their patient's presenting tumor type [32, 33]. While MIPOGG considers all known CPSs, it acts as a screening tool as opposed to a formal "risk calculator" like the models presented in the previous section. Other models similar to the MIPOGG app are currently being developed and trialed. One of these models, the TuPS model, includes 2D and 3D photographs to evaluate dysmorphic features, which are present in some CPSs [34]. This, however, requires a review from health professionals who have expertise in evaluating dysmorphisms and linking them to certain CPSs. Other types of eHealth tools evaluate specific

family history features in patients diagnosed with particular adult-onset cancer types like breast cancer or colorectal cancer [35].

The integration of artificial intelligence (AI) technologies within current eHealth tools, such as MIPOGG or tools that include photographs, will likely increase the power when determining the likelihood for a patient to have a CPS. Machine learning technologies can help identify links and strengths of associations between phenotypic features and genotypes in people with cancer. By including the pattern recognition capabilities of these new technologies, the yield of CPS detection can be increased compared to relying solely on physician recognition or the current knowledge used to build eHealth tools. However, in order to establish this recognition and successfully integrate AI into the development and testing of such models, a massive amount of structured patient-level data are needed.

By rationalizing the recognition process and providing a resource which attempts to cover all pediatric CPSs, being referred to genetics is no longer dependent on individual physician knowledge about tumor-CPS associations. Nevertheless, using an eHealth approach to make a determination about genetic referral assumes a certain structure to the healthcare system and access to a genetics service. In many countries, accessing a genetics clinic is challenging, if it's even an option at all. There may also be dramatic differences in access between more urban and rural centers within the same country. The universal testing approach does not mitigate the problem, as a lack of accessible genetics services makes the process of testing all-comers with pediatric cancer extremely challenging and often unfeasible. Additionally, the cost of such an approach may be prohibitive depending on the country and healthcare payer structure. While none of the approaches described will address the needs of all patients and their families around the world, it is important to consider how each option could better serve more patients, in a more equitable way, within a given healthcare system and by a given provider.

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