Familial Cancer (2013) 12:145–157
DOI 10.1007/s10689-013-9637-8

ORIGINAL ARTICLE

The History of Lynch Syndrome

C. Richard Boland · Henry T. Lynch

Published online: 2 April 2013

The early years: 1895–1937

A. S. Warthin and Family G

One hundred years ago, Aldred Scott Warthin, MD, PhD, [1] Chairman of the Department of Pathology at the University of Michigan in Ann Arbor, reported the first family with the disease we now call Lynch Syndrome (Fig. 1). In 1895, a woman who worked as his seamstress reported distress over the fact that many family members over several generations had succumbed to cancer, and she feared the same for herself. Indeed, she developed endometrial cancer, and died of that disease as she predicted. Warthin dryly noted that "the statistical study of carcinoma … [has] been carried as far as it can be profitable; and certainly but little that is new has been gained by this method during the last decade". Throwing a statistical approach to the wind, he undertook a "fairly complete survey" of the family, and created a pedigree, showing which family members had developed cancer, and their relationships (Fig. 2).

The seamstress' immediate sibship included 10 members; 2 had uterine cancers, 2 had stomach cancers, and one had an "abdominal cancer". The descendents of all 5 of those with cancer also had multiple cancers. Among those in the family without cancer who had children, none of the progeny had developed cancer. Warthin concluded that there could be, at least in this instance, a familial predisposition to cancer. The family had emigrated from Germany to Michigan before the Civil War; Warthin called them "Family G".

Warthin also reported that, in 3,600 cases of neoplasia that had come through his laboratory at the University of Michigan from 1895 to 1912, 1,600 of these were carcinomas, and that "about 15 %" of those had a family history of carcinoma. Reinforced by a report in 1912 from a German investigator named Levin, Warthin concluded that there were "cancerous fraternities", and that there was "some influence of heredity on cancer". He presented a series of pedigrees to illustrate his case.

Warthin [2] wrote a "further study" of cancer family "G" in 1925. By now, he had concluded that the familial susceptibility to cancer was particularly true for carcinoma of the gastrointestinal tract and uterus. He recognized the early age of onset of the cancers, and suggested that the tumors might be occurring "at an earlier age in successive generations"—a phenomenon we now call anticipation, but which has not been substantiated in further studies of Lynch Syndrome. He also noted that 3 young members of the family presented with appendicitis, but at operation were found to have advanced cancer—presaging the proximal colonic tendencies for the colorectal cancers (CRCs) in this disease. Among 146 family members, almost 32 % had developed cancer, at a median age of 37.9 years. He also commented that his observations had been met "with little favor among surgical writers". (Some things never change.)

Warthin died in 1931, but his colleagues Hauser and Weller [3] issued a "further report on the cancer family of Warthin" in 1936. As more time passed, and more

C. R. Boland (✉)
GI Cancer Research Laboratory (250 Hoblitzelle), Baylor University Medical Center, 3500 Gaston Avenue, Dallas, TX 75246, USA
e-mail: rickbo@baylorhealth.edu

H. T. Lynch
Department of Preventive Medicine, Creighton University, 2500 California Plaza, Omaha, NE 68178, USA
e-mail: htlynch@creighton.edu

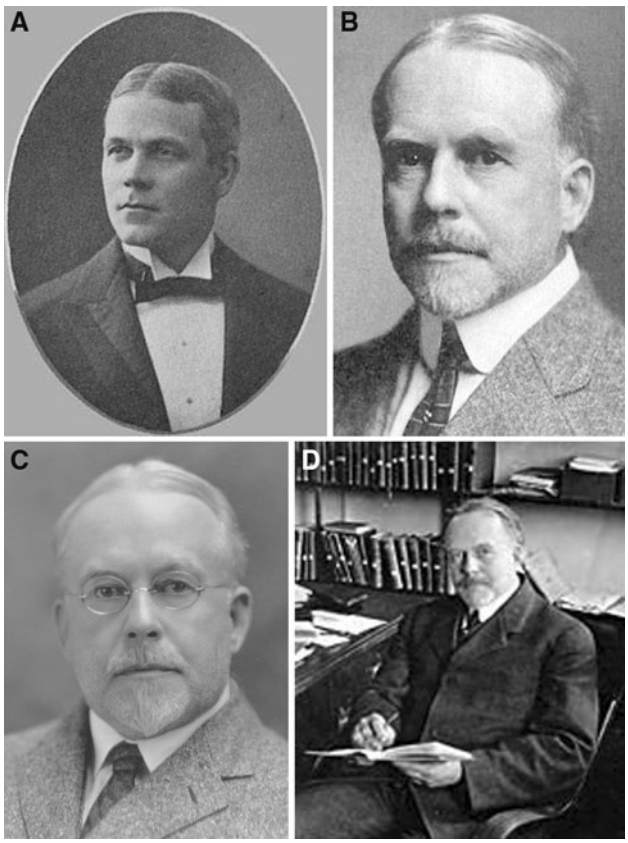


Fig. 1 Photos of A. S. Warthin, M.D., Ph.D. **a** Photo of Warthin as a young man, about 1900. **b** Formal photo of Warthin, date uncertain. **c** Informal photo of Warthin, date uncertain [16]. **d** Warthin at his desk, date uncertain

individuals were followed for a longer period, mitigating one type of ascertainment bias, the average age for death from cancer rose to 48.3 years. They noted that there were no cases of cervical cancer among those with uterine cancer, that there were many gastrointestinal cancers, and few breast cancers. They provided detailed pathological analyses of the tumors from each branch of the family. As more data accumulated, they concluded that there was a "diminishing incidence" of cancer with successive generations. These authors proposed that this family provided more evidence for an "inheritable organ-specific predisposition to carcinoma".

Twentieth century insights

From Warthin to Lynch

The story grew cold during the period from 1937 until the 1960s. Occasional case reports of this disease came from the Mayo Clinic in 1941 [4], England in 1956 [5], and a variety of locations in the 1960s [6–11]. None the less, the existence of a familial form of CRC that was not familial adenomatous polyposis remained in considerable doubt. One person who noted these familial clusters of cancer was Henry T. Lynch, MD, who reported several families in detail in 1966–1967 [12–14] (Fig. 3). Lynch was aware of Warthin's "Cancer Family G"; therefore, he arranged for a family reunion near Ann Arbor, MI, to learn more about this family. He conducted a detailed medical genetic investigation of the family, obtained data on >650 family members (among whom 95 had now developed cancers), and found a predominance of cancers of the colon, uterus and stomach in the kindred in the iconic "Cancer Family 'G' Revisited" manuscript of 1971 [15] (Fig. 4). Once more, progeny of affected individuals continued to be at risk for early-onset cancers. He recognized the autosomal dominant nature of inheritance. A variety of hypotheses were proposed to explain the disease, but the time for discovery of the basis of hereditary cancer had not yet arrived. He used the term "Cancer Family Syndrome" in this report.

This would not be the final report on Cancer Family G. In 2005, Douglas et al. [16] (from the University of Michigan) provided additional confirmatory follow-up of the family with data on 929 descendants of the original progenitor, and reported on the specific mutation in the *MSH2* gene. This work verified the risks for cancer of the colon and endometrium, showed that the risks for gastric cancer which were initially prominent had disappeared through the 20th century, and provided standardized incidence ratios for cancers of various organs. There is probably no other instance in which one family has contributed so much to the understanding of an important genetic disease such as this.

Giving "cancer family syndrome" a more specific name

In 1973, C. Richard Boland, MD wrote a medical school thesis entitled "A Familial Cancer Syndrome", recognizing the same disease; this led to the publication of 2 papers describing additional families with Lynch Syndrome. In the first of these [17], the term "Cancer Family Syndrome" was used, based upon Lynch's nomenclature. However, when a second family was reported later, it was noted that some families had a phenotype with only CRC, whereas other families had the characteristic non-colonic cancers we now recognize in this disease. The terms Lynch Syndrome I and II were used for the first time to distinguish those families with a CRC-only versus the full spectrum of cancers [18]. There is now evidence that at least some germline mutations can produce a CRC-predominant syndrome [19], although the designations of Lynch Syndrome I and II are no longer used or necessary. Interestingly, in

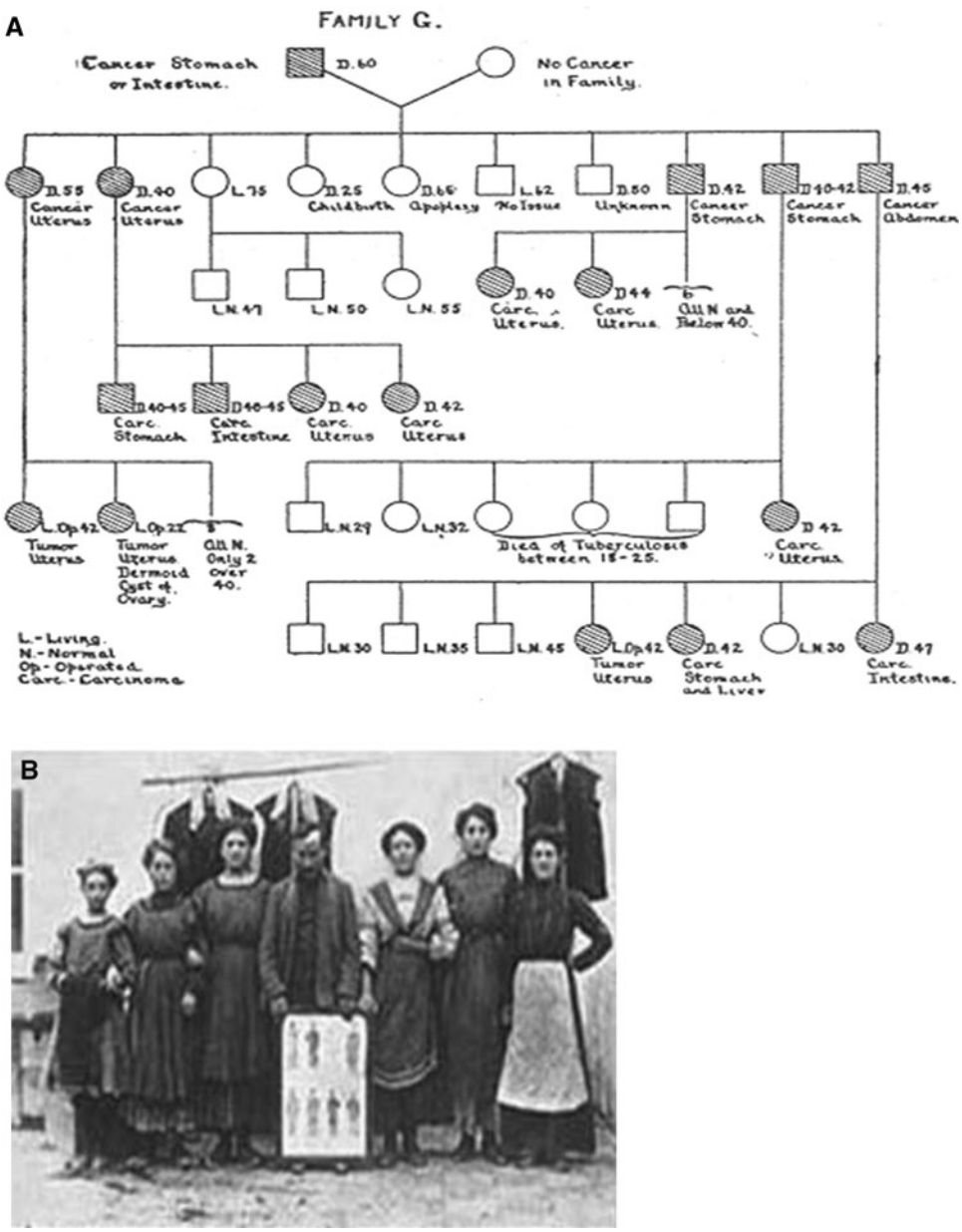


Fig. 2 **a** Warthin's initial pedigree of Cancer Family G, from his seminal article in the Arch Int Med, 1913 [1]. Note the predominance of uterine and gastric cancers in the earlier generations. **b** Photo of the immigrant founder of Family G. He had 10 children, 6 of whom developed cancer. He died in 1856 at age 60, of cancer. By the third generation, there were 70 descendents, 33 of whom had developed uterine, gastric or colon cancer

1985, Lynch first used the term "hereditary non-polyposis colorectal cancer" or HNPCC for this disease, which was the accepted term for many years [20, 21]. It would not be until the genetic basis of the disease was discovered, and more importantly, the recognition that not all familial clusters of CRC represented one disease, that the term Lynch Syndrome was finally applied to those families with germline mutations in DNA mismatch repair (MMR) genes [22].

The Amsterdam criteria

In this time frame, Hans Vasen, MD, from the Netherlands, emerged as a major contributor to the field. Hans was one of several key members in the formation of the "International Collaborative Group on Hereditary NonPolyposis Colorectal Cancer" (or ICG-HNPCC), which was conceived during a CRC meeting in Jerusalem in 1989, and had its first formal meeting in Amsterdam in 1990 [23]. Meetings were held on a regular basis thereafter, particularly as the understanding of hereditary CRC grew, and the biological basis of the disease was uncovered.

While some observers doubted the existence of a hereditary non-polyposis CRC, Hans and other interested clinicians accumulated and characterized familial clusters of CRC, and developed the "Amsterdam Criteria", which were valuable for finding families who had Lynch Syndrome [24, 25]. Gathering "reagent grade" families for

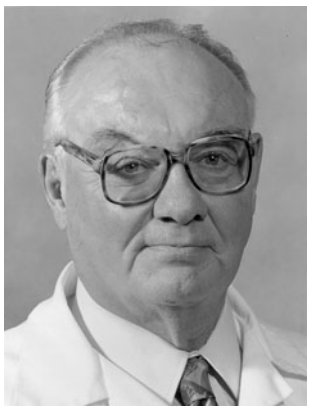


Fig. 3 Henry T. Lynch, M.D

analysis, and the concomitant evolution of molecular genetics, would soon lead to the discovery of the genetic basis of Lynch Syndrome [26]. Ultimately, the ICG-HNPCC merged with the Leeds Castle Polyposis Group [27] to form the "International Society for Gastrointestinal Hereditary Tumours", or InSiGHT, which continues to have semi-annual meetings and research initiatives (see www.insight-group.org).

Microsatellite instability and CRC

From the late 1960s until 1992, progress in understanding Lynch Syndrome was slow. The clinical features were refined, but there was no premalignant phenotype, and only a few clues about the nature of the tumors that could lead to a genuine understanding of the disease. There were multiple attempts to understand the basic mechanism responsible for the disease; all failed.

In 1989–1990, the laboratory of Bert Vogelstein, MD proposed that colorectal neoplasia developed through multistep carcinogenesis, and that the sequential loss of specific fragments of chromosomal DNA was a key part of this process. Loss of DNA in a tumor was termed "loss of heterozygosity" or LOH, because of the genetic techniques used to detect it. Many laboratories, including that of Manuel Perucho, PhD, were looking for LOH in cancer tissues as these would represent presumptive loci for tumor suppressor genes involved in colorectal carcinogenesis. Perucho used a technique called "arbitrarily primed PCR" to amplify randomly selected genetic targets from paired samples of CRC and normal tissues. The PCR products were separated by gel electrophoresis, and compared side-by-side to look for a genetic deletion in the cancer DNA compared to its normal counterpart [28]. There were plenty of these deletions to be found, but he noted that there were also subtle changes in the lengths of some of the amplified DNA fragments in tumor tissues, specifically those that happened to contain simple repetitive sequences called

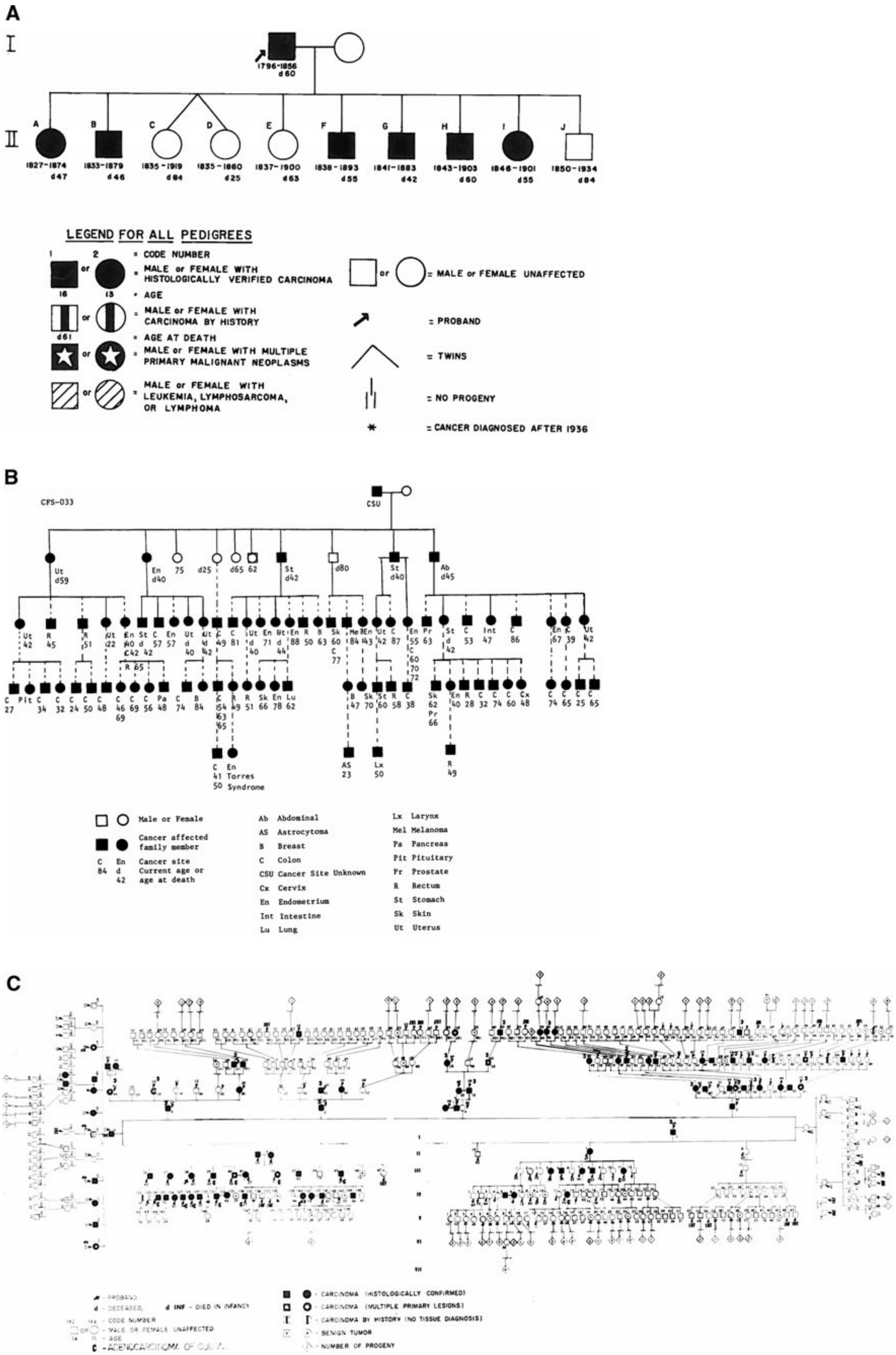
Fig. 4 **a** "Cancer Family G Revisited", pedigree from the 1971 article by Lynch that updated the index family with follow-up on 650 family members [15]. **b** As Lynch added information, the data on subsequent cancers increased with each successive generation, as noted. **c** Lynch continued with the pedigree, annotating additional tumors over time, resulting in this circular pedigree

"microsatellites" [29] (Fig. 5). Only some CRCs showed this, but those that did had an estimated 100,000 such mutations. He proposed that this subset of CRCs was different from the rest, and that this represented a unique "pathway" through which colorectal tumors could evolve. He had considerable difficulty getting this revolutionary idea published—although it did finally appear in *Nature*, after some delay. While he was sending his manuscript around for approval, two other laboratories also discovered microsatellite instability (MSI). One was the lab of Stephen Thibodeau, PhD, from the Mayo Clinic, who also noted that MSI was mainly seen in CRCs from the proximal colon, that survival was better in this group, and that the presence of MSI correlated inversely with the LOH events described by Vogelstein's group. He suggested that this might represent a novel mechanism compared with other CRCs [30].

At approximately the same time, an international consortium that included Vogelstein from Johns Hopkins, together with Albert de la Chapelle, Lauri Aaltonen and Paivi Peltomaki from Finland (and others who provided the appropriately identified families), were using microsatellite markers in an extensive linkage analysis study on familial clusters of CRC. On one afternoon in the spring of 1993 (specifically 3:45 PM on Saturday March 13), Lauri Aaltonen identified significant linkage for Lynch Syndrome on chromosome 2p, using the microsatellite marker, D2S123 (which was the 345th marker analyzed in this study) [31]. The move from complete darkness to light occurred with an astonishingly quick stroke of discovery. The presumption was that a tumor suppressor gene was in the vicinity of D2S123, and the logical experiment was to look for LOH in the CRC tissue from an affected patient. Instead of LOH, they found MSI [32] (Fig. 6). The 3 papers (one from Thibodeau and two from the international consortium) all appeared in the same issue of *Science* on May 7, 1993. The entire world of hereditary CRC was turned upside down, as there was, for the first time, a clue regarding the molecular basis of this disease. Perucho, who had initially noted MSI and proposed a separate pathway, had his paper published a few weeks later, on June 10, 1993 [29], quite unhappy about the delays produced by certain journal editors.

From MSI to DNA MMR genes

The speed of discovery increased substantially from that point. Interestingly, none of the initial discoverers of MSI recognized exactly how the autoradiograms they had


A
I
1796-1856
d60
II
1827-1874 d47
1833-1879 d46
1835-1919 d84
1835-1860 d25
1837-1900 d63
1838-1893 d55
1841-1883 d42
1843-1903 d60
1846-1901 d55
1850-1934 d84
LEGEND FOR ALL PEDIGREES
= CODE NUMBER
= MALE or FEMALE WITH HISTOLOGICALLY VERIFIED CARCINOMA
= AGE
= MALE or FEMALE WITH CARCINOMA BY HISTORY
= AGE AT DEATH
= MALE or FEMALE WITH MULTIPLE PRIMARY MALIGNANT NEOPLASMS
= MALE or FEMALE WITH LEUKEMIA, LYMPHOSARCOMA, OR LYMPHOMA
= MALE or FEMALE UNAFFECTED
= PROBAND
= TWINS
= NO PROGENY
= CANCER DIAGNOSED AFTER 1936
B
CFS-033
CSU
C 41 50
En Torres Syndrome
Male or Female
Cancer affected family member
Cancer site
Current age or age at death
Ab Abdominal
AS Astrocytoma
B Breast
C Colon
CSU Cancer Site Unknown
Cx Cervix
En Endometrium
Int Intestine
Lu Lung
Lx Larynx
Mel Melanoma
Pa Pancreas
Pit Pituitary
Pr Prostate
R Rectum
St Stomach
Sk Skin
Ut Uterus
C
PROBAND
d INF - DIED IN INFANCY
CARCINOMA (HISTOLOGICALLY CONFIRMED)
CARCINOMA (MULTIPLE PRIMARY LESIONS)
CARCINOMA BY HISTORY (NO TISSUE DIAGNOSIS)
BENIGN TUMOR
NUMBER OF PROGENY

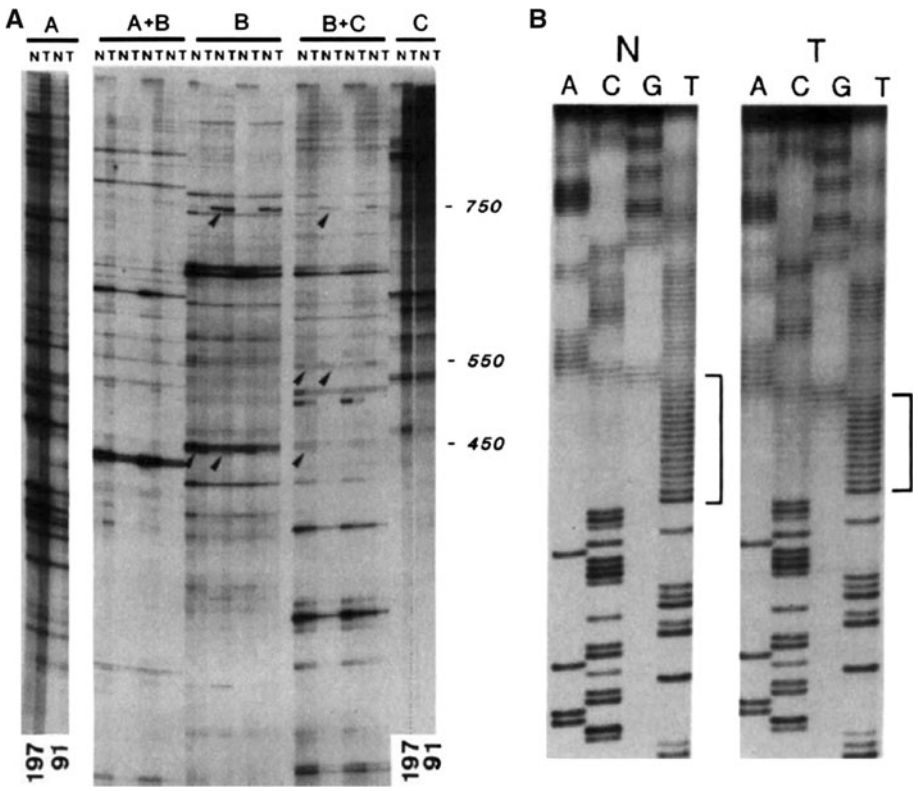


Fig. 5 Discovery of MSI. **a** The laboratory of Manuel Perucho, PhD was hunting for allelic losses and gains in colorectal cancer tissues using arbitrarily primed PCR, published in 1992 [28]. This was a powerful technique for finding copy number changes and LOH as they ran the autoradiograms from cancer tissues and normal colon samples side-by-side looking for bands present in normal tissue that were fainter or stronger in the cancer. In 1993, they detected small deletions in the PCR products, as indicated by the arrowheads, using several different arbitrary primers and primer pairs (listed as primers A, A + B, B, B + C, and C, along the *top*) on DNA from normal colons (N) and matched samples of tumor (T) [29]. **b** The novel bands were cut from the original gels and sequenced. The altered bands contained mononucleotide repeats (a poly-T microsatellite sequence, indicated by the *brackets*), and the DNA from the tumor (T) harbored deletions in the repeat tract

produced were the key to understanding the disease. Laboratories studying genetics in bacteria and yeast had previously discovered the DNA MMR system, and knew that if MMR genes were inactivated by mutation in microorganisms, it resulted in widespread mutations at microsatellite sequences.

Several laboratories entered into a race to clone the human homologs of these genes, and determine if there were germline mutations in DNA MMR genes in families with Lynch Syndrome. The first to do this successfully was the laboratory of Richard Kolodner, PhD, who was an established investigator in yeast genetics, and had identified the *MSH2* gene in yeast, but had not previously ventured into human disease or cancer. On December 3, 1993, less than 6 months after the initial linkage of MSI with hereditary CRC, this lab, together with several collaborators, cloned the human homolog of the DNA MMR gene *MSH2*, and found a sequence variation in a family with Lynch Syndrome that was present in those who had developed cancer [33]. Even more astonishing, just 2 weeks later, on December 17, 1993, the international consortium led by Vogelstein and de la Chapelle found 3 additional kindreds with inactivating mutations in the human *MSH2* gene [34]. Moreover, they identified a CRC cell line, HCT116, that had MSI, and this created the first in vitro model in which to study the basics of the process [35]. For those interested in Lynch Syndrome, this was like the first step on the moon.

Earlier in 1993, a Lynch Syndrome family had been characterized in Sweden, but in this instance the genetic linkage pointed to chromosome 3p, rather than 2p, where *MSH2* had been found [36]. This launched yet another race to identify the gene. Again, the Kolodner group cloned the human *MLH1* gene, in collaboration with R. Michael Liskay, PhD, who as well as Kolodner had been working on MutL-related genes in yeast, and reported germline mutations in additional Lynch Syndrome families on March 17, 1994 [37]. Not to be outdone, the international consortium reported the same, on March 18, 1994 [38]. They also found that *MLH1* (rather than *MSH2*) was mutated in the HCT116 cell line. By September, 1994, the human *PMS2* and *PMS1* genes were also cloned, and linked to Lynch

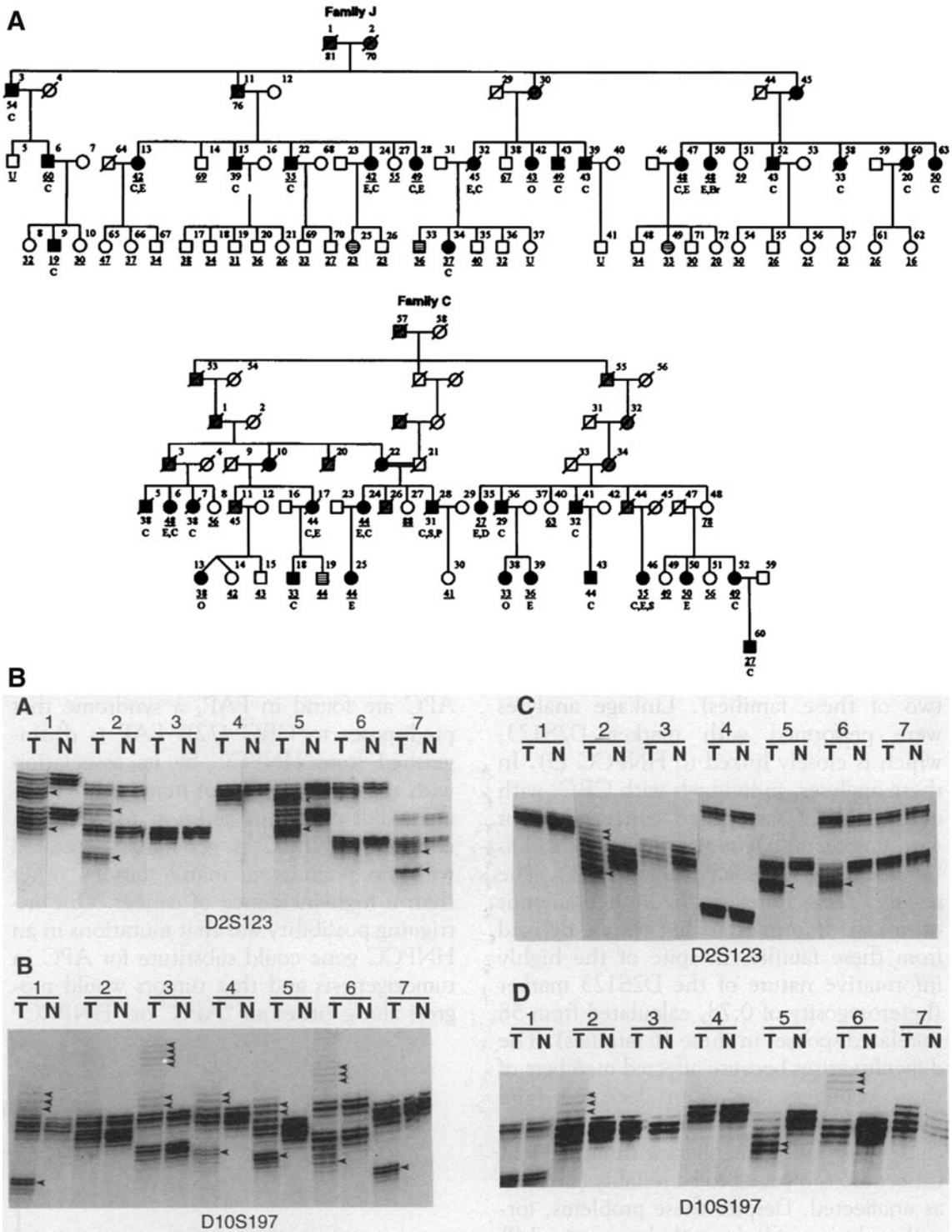


Fig. 6 Lynch Syndrome kindreds are linked to chromosome 2p and MSI. **a** In 1993, the international consortium organized by Vogelstein, de la Chapelle and others had identified familial CRC kindreds as shown in the pedigrees. Two had significant linkage between CRC and the microsatellite marker D2S123, located on chromosome 2p [31]. **b** The investigators used the microsatellite marker D2S123, looking for LOH in the CRC tissue DNA. Instead of LOH, they found MSI as shown by the anomalously migrating bands indicated by the *arrowheads* [32]

Syndrome [39]. So, in an incredible period of about 16 months, Lynch Syndrome was firmly put on the scientific map, linked to MSI, which led to the identification of the human DNA MMR genes, and it was possible to think about developing tests to diagnose the disease. Over the next several years, it was found that *PMS1* was not actually

Fig. 7 Key contributors to the field, in the modern era of molecular biology and genetics. **a** Hans Vasen, MD (Leiden University, The Netherlands). **b** Manuel Perucho, PhD (Sanford-Burnham Medical Research Unit, La Jolla, CA). Discovered MSI, and proposed a novel pathway for CRC development. **c** Stephen Thibodeau, PhD (Mayo Clinic, Minnesota). Independently discovered MSI, and suggested that these tumors evolved through a unique mechanism that did not involve LOH events. **d** Bert Vogelstein, MD (Johns Hopkins University). Linked MSI to hereditary colorectal cancer; identified several of the DNA MMR genes, and linked mutant MMR genes to Lynch Syndrome. **e** Albert de la Chapelle, MD, PhD (The Ohio State University). Together with Vogelstein and others, linked MSI to hereditary colorectal cancer; identified several of the DNA MMR genes, and linked mutant MMR genes to Lynch Syndrome. **f** Lauri Aaltonen, MD, PhD (University of Helsinki, Finland). Together with Vogelstein and de la Chapelle, made the critical observation that hereditary CRC was linked to a locus on chromosome 2p, and that there was MSI in the linkage marker, which was a microsatellite sequence. **g** Richard Kolodner, PhD (University of California, San Diego). Cloned the human homologs of *MSH2* and *MLH1*, and found germline mutations in families with Lynch Syndrome. **h** Minoru Koi, PhD (Baylor University Medical Center, Dallas, TX). Created the first in vitro models of Lynch Syndrome from the HCT116 cell line, using stable chromosome transfer to correct the DNA MMR defect. **i** C. Richard Boland, MD (Baylor University Medical Center, Dallas, TX). Studied hereditary colon cancer as a medical student, coined the term "Lynch Syndrome", used the cell model developed by Koi in a series of studies on the response of DNA MMR deficient cells to chemotherapeutic drugs. **j** Sir John Burn, MD, reported the first effective medical intervention for Lynch Syndrome—aspirin

a Lynch Syndrome gene, and *MSH6* was brought into the fold as the fourth Lynch Syndrome gene, first by its involvement in cell lines with MSI [40, 41], and finally via germline mutations in affected patients with different types of family histories of cancer, often with later onset than seen in classic Lynch Syndrome [42].

Evolution of diagnostic tests for Lynch Syndrome: from Amsterdam, through Bethesda, to Jerusalem

The ability to determine which patients and families actually had Lynch Syndrome permitted a refinement of the diagnostic approaches during the last decade of the 20th century. Antibodies were developed to the DNA MMR proteins MSH2, MSH6, MLH1 and PMS2, and the diagnostic approach to MSI was standardized in an NCI-sponsored Workshop in Bethesda in November, 1997. The Workshop manuscript reported a standardized diagnosis and panel of microsatellite markers, and the published paper has been cited >2,000 times [43]. This manuscript also developed and reported the "Bethesda Guidelines", which were intended to identify CRC tissues that should be targeted for analysis, either looking for MSI or abnormal immunohistochemistry (IHC). The Bethesda Guidelines were revised in 2004 [44], much as the original Amsterdam Criteria [24] had been in 1999 [25]. All of these recommendations have been supplanted by our current understanding that many true Lynch Syndrome families do not meet the Amsterdam Criteria (initially intended to identify families from whom the genes could be found), that many individuals with Lynch Syndrome do not meet the Bethesda Guidelines, and conversely, many who meet these criteria or guidelines do not have germline mutations in any DNA MMR gene [45]. This problem ultimately led to a workshop in Jerusalem in 2010, in which it was recommended that any CRC in a person <70 years old should be screened by MSI testing or IHC for possible Lynch Syndrome [46]. Photos of many—but certainly not all—of the key contributors to the field are shown in Fig. 7.

Lynch Syndrome in the 21st century

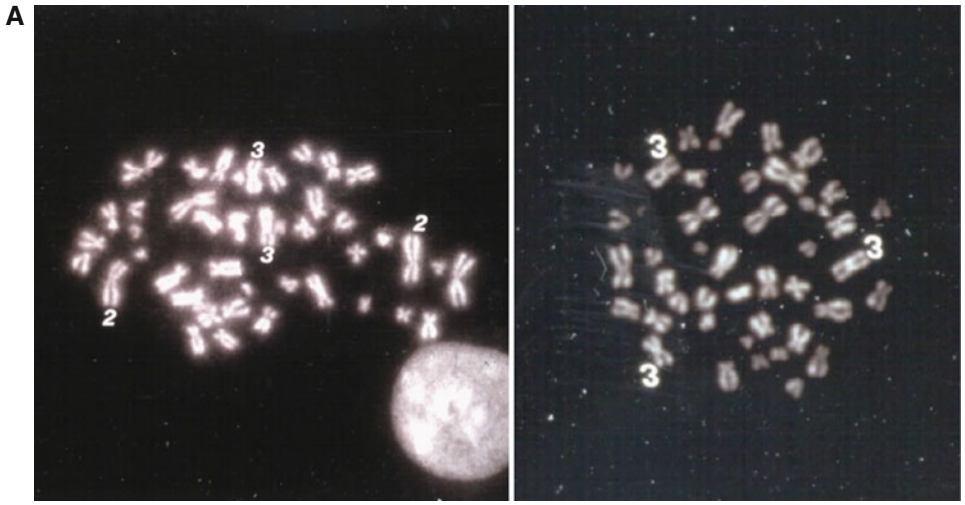
"Syndrome X"

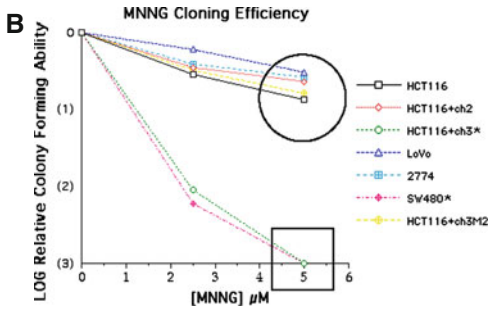
The identification of the genes responsible for Lynch Syndrome gave those working in the field a euphoric sense that the problem had been substantially solved. However, as more details emerged, it became clear that only the surface had been scratched, and there was much more to be learned about the disease. First, it had been suspected (perhaps naively) that once the genes causing Lynch Syndrome were identified, we would be able to characterize all familial clusters of CRC. The Colon Cancer Family Registry (C-CFR), a large international consortium of groups, collected 3,422 individuals from 161 families that met the Amsterdam Criteria between 1997 and 2001. DNA from each family was subjected to the best available efforts to find germline mutations in DNA MMR genes. Only 60 % of these families had a germline mutation (i.e., actually had Lynch Syndrome). The other 40 % had CRCs that did not have MMR deficiency. Therefore, they did not have Lynch Syndrome [22], and a new disease was identified: Familial Colorectal Cancer-Type X [45]. These families had a lower penetrance for CRC, later onset of the cancers, and did not have an increase in the non-colonic tumor spectrum seen in Lynch Syndrome. There are many genetic diseases such as Peutz-Jeghers Syndrome, Juvenile Polyposis, Cowden's Disease, Li-Fraumeni Syndrome, and others that experience an increase in risk for CRC, but "Syndrome X" does not appear to fall into any of those groups, and remains an important research challenge at this time [47].

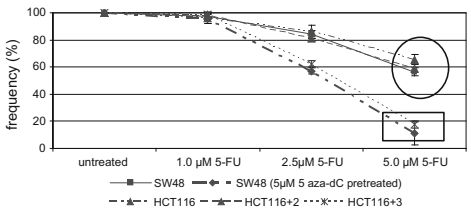
Interpreting the genetic analyses of the DNA MMR genes

Next, as more data came in and genetic tests became widely available commercially, it became apparent that it was not always simple to determine which DNA sequence variations in the DNA MMR genes cause Lynch Syndrome, and which are innocent sequence polymorphisms. Premature stop codons were easy to interpret, but many of the sequence variations altered gene splicing sites (not too hard to interpret once the "rules" were learned), or missense mutations, which changes the amino acid in that position in the encoded protein. It is not always possible to predict changes in protein folding and function based upon the change in the amino acid sequence alone. So, many genetic tests returned with clinically uncertain or uninterpretable results [48]. This created new challenges for the clinician and genetic counselors.

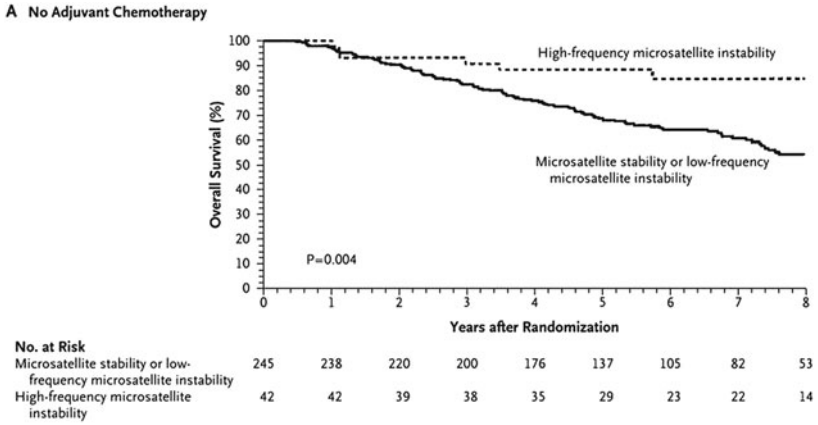
One of first insights occurred when it became apparent that the *MSH2* gene was often mutated by large deletions that were not detectable using the standard sequencing techniques. Over one-third of the mutations in this gene were responsible for inactivating mutations in a key Dutch study, and they accounted for a substantial proportion (>6 %) of all Lynch Syndrome in their registry [49]. One approach to identify large genomic deletions was to separate the paternal and maternal alleles for individual analysis—a labor-intensive technique called "conversion to haploidy", but this was not widely embraced [50]. Eventually, techniques became available that permitted an estimation of the number of alleles present at each exon (multiplex ligation-dependent probe amplification, or MLPA), which permitted the detection of large genomic deletions, and helped resolve this confusion.

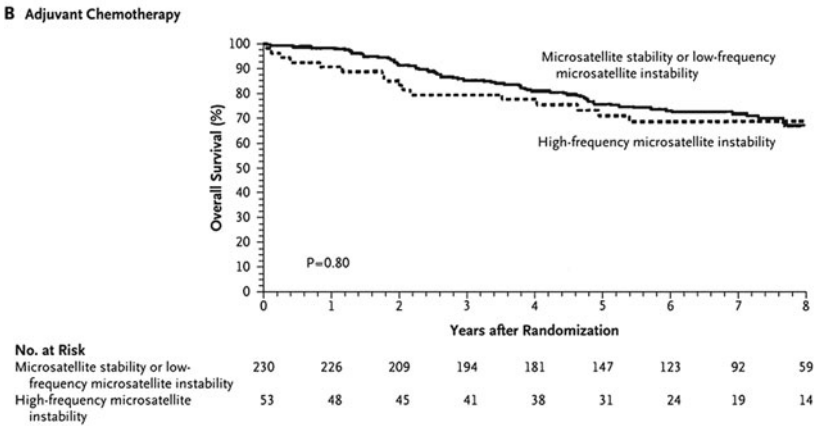

A
3
2
3
2
3
3
3


B
MNNG Cloning Efficiency
LOG Relative Colony Forming Ability
0
(1)
(2)
(3)
0 1 2 3 4 5 6
[MNNG] μM
HCT116
HCT116+ch2
HCT116+ch3*
LoVo
2774
SW480*
HCT116+ch3M2


C
frequency (%)
100
80
60
40
20
0
untreated
1.0 μM 5-FU
2.5μM 5-FU
5.0 μM 5-FU
SW48
SW48 (5μM 5 aza-dC pretreated)
HCT116
HCT116+2
HCT116+3

D


A No Adjuvant Chemotherapy
Overall Survival (%)
High-frequency microsatellite instability
Microsatellite stability or low-frequency microsatellite instability
P=0.004
Years after Randomization
No. at Risk
Microsatellite stability or low-frequency microsatellite instability 245 238 220 200 176 137 105 82 53
High-frequency microsatellite instability 42 42 39 38 35 29 23 22 14


B Adjuvant Chemotherapy
Overall Survival (%)
Microsatellite stability or low-frequency microsatellite instability
High-frequency microsatellite instability
P=0.80
Years after Randomization
No. at Risk
Microsatellite stability or low-frequency microsatellite instability 230 226 209 194 181 147 123 92 59
High-frequency microsatellite instability 53 48 45 41 38 31 24 19 14

◀ **Fig. 8** DNA MMR deficiency and response to chemotherapeutic agents. **a** In early 1994, Minoru Koi, PhD created a DNA MMR-corrected model by stably transferring a copy of human chromosome 3 into the *MLH1*-deficient CRC cell line HCT116, which is diploid. On the *left* is a karyotype of the uncorrected cell line, with 2 copies of both chromosomes 2 and 3; this cell line is DNA MMR deficient and has MSI. On the *right* is HCT116 + chr3, which has 3 copies of chromosome 3, the MSI has been corrected, and the cell line is MMR proficient [52, 53]. **b** The CRC cell lines that are MMR deficient (as shown in the *circle*) can tolerate increasing doses of the alkylating agent MNNG, which damages DNA, as shown in the cells that continue to have high cloning efficiency (HCT116, HCT116 + chr2, LoVo, 2774, and HCT116 + chr3M2). However, the cell lines that are DNA MMR proficient (shown in the *square*) are sensitive to DNA damage, and cannot be grown in 5 μM MNNG (the lower 2 lines are HCT116 + chr3 and SW480) [54]. **c** The MMR-deficient cell line SW48 has undergone methylation-induced silencing of *MLH1*, which is reversed by the demethylating drug 5azadC. The *upper curves* (in the *circle*) show that DNA MMR-deficient cells (SW84, HCT116, HTC116 + chr2) can tolerate up to 5 μM 5-FU, but MMR-proficient cells, such as HCT116 + chr3 or SW48 after demethylation) cannot (shown in the *rectangle*). This in vitro experiment suggested that MMR-deficient tumors may not have the same response to chemotherapy as MMR-proficient ones [54, 55]. **d** In 2003, a retrospective study of CRC patients prospectively randomized to receive either 5-FU-based adjuvant chemotherapy or not, analyzed outcomes according to MSI status. The *upper panel* shows that patients with MSI CRCs had a better outcome than those without MSI if they did not receive chemotherapy. However, those randomized to receive 5-FU-based chemotherapy lost the survival benefit based upon MSI, and the two survival curves collapsed, with slight increase in mortality among the treated patients [61]

A second insight into perturbations in the *MSH2* gene was the discovery that deletion of the stop codon of the *EPCAM* gene, which is immediately upstream of the *MSH2* gene, resulted in epigenetic silencing of *MSH2*. Therefore, although no germline mutation was present in the *MSH2* gene, the alteration in *EPCAM* created a "heritable somatic inactivation" of *MSH2* in all tissues that expressed *EPCAM* [51]. By finding this mutation in a large kindred, it was found that this situation creates a CRC-predominant form of Lynch Syndrome, reminiscent of Lynch Syndrome I predicted some 25 years earlier [18].

Adjuvant chemotherapy and Lynch Syndrome

Shortly after the discovery of the *MSH2* and *MLH1* genes in 1993–1994, Minoru Koi, PhD created the first laboratory model to study the biology of DNA MMR-deficient cells in vitro by stably transferring a copy of human chromosome 3 into HCT116 CRC cells, correcting the loss of *MLH1* [52] (Fig. 8). Subsequent experiments led to the conclusion that DNA MMR-deficient cells were intrinsically resistant to DNA damage, similar to microbial cells with inactivating mutations in these genes [53, 54]. The implications were that certain chemotherapeutic drugs might not be fully effective against MSI cancers. Additional experiments showed that MMR-deficient cells were resistant to 5-fluorouracil (5-FU), the mainstay of adjuvant chemotherapy for Stage III CRC [55]. Resistance was found for other chemotherapeutic drugs [56, 57], and identical drug resistance was found in CRC cells with acquired methylation-induced silencing of *MLH1* [58].

It was therefore necessary to determine whether patients with MSI CRCs were refractory to conventional chemotherapy in a clinical study. The first published report on the subject suggested that patients with MSI CRCs had a "striking survival benefit" when given adjuvant chemotherapy [59]. However, a serious design flaw had led to an erroneous conclusion. The study was retrospective, and the patients had not been randomized to receive chemotherapy; rather, they had been selected by their oncologists to either receive treatment or not, presumably on the basis of their age and/or performance status. In fact, the entire group of the patients selected for chemotherapy had a better 5 year survival whether they were treated or not. However, 64 % of the CRC patients <68 years old had been selected for treatment versus only 19 % of those ≥68. None the less, this report created a problem in which the empirical observations were at odds with what had been predicted from the in vitro biology of the tumors.

This initial finding was not supported by 11 subsequent studies on the subject (reviewed in detail in [60], Table 3). The first contrary paper was a multicenter collaboration of patients enrolled in randomized trials, and they found that patients with tumors showing MSI had substantially better overall 5 year survivals, and this was particularly so *if they did not* receive adjuvant chemotherapy. Even worse, there were non-significant trends towards increased cancer-related mortalities in the Stage II and Stage III groups given adjuvant drug treatment [61].

Subsequent studies have raised the possibility that the improved survival in CRC patients with MSI may not be a sole consequence of the intrinsic resistance to chemotherapy. CRCs with MSI contain substantially more tumor-infiltrating lymphocytes, and it has been proposed that this brisk immune response may be responsible for limiting the spread of these tumors [62, 63]. Thus, the actual mechanism responsible for the poor response to chemotherapy may be related to the immune response to the hypermutated tumor cells, rather than—or in addition to—intrinsic resistance to the therapy.

Aspirin and Lynch Syndrome

The most recent chapter in Lynch Syndrome has opened the door to an entirely new phase of managing cancer risk. Sir John Burn of Newcastle, UK, designed a prospective, randomized, placebo-controlled, multicenter study of the impact of aspirin and fiber on the development of neoplasia in Lynch Syndrome. The initial study design was to

determine whether 600 mg of aspirin, 30 g of a fermentable dietary fiber ("resistant starch"), or both, might suppress the formation of adenomatous polyps in the colon over a period of 4 years. Neither intervention had any effect on polyp recurrence; in fact, the relative risk (RR) for subsequent adenomatous polyps was exactly 1.0 [64].

Although a less persistent man might have been deterred, Burn was not. He conducted a follow-up analysis of outcomes after another 4 years, and discovered a significant reduction in the risk of CRC, both in those randomized to aspirin (RR for CRC = 0.63, by intention to treat), and to those who actually took the aspirin for at least 2 years (RR for CRC = 0.41, per protocol) [65]. No beneficial effect was derived from the supplemental dietary fiber. No excess in adverse events was seen in the aspirin-treated patients. This remains to be confirmed, and the optimal dose of aspirin has not been determined; but this represents a game-changing event in the history of hereditary cancer in general.

Conclusions

Lynch Syndrome was initially recognized by the University of Michigan pathologist A.S. Warthin in 1913, who listened to his seamstress, gathered an extensive family history, constructed a pedigree, and proposed a familial, and perhaps genetic, explanation for this "cancerous fraternity". He followed up his own work, and others followed, finding numerous similar families in a variety of communities and countries. The coordinated identification of rigorously defined families led to the linkage of the cancer-prone phenotype to a single locus on chromosome 2p in 1993. A serendipitous experiment permitted the prepared investigators to recognize that some familial CRCs were associated with a novel cancer "pathway" that had been independently discovered by 2 other investigators who did not suspect that there might be a familial form of this pathway. A focused (and furious) race ensued that led to the discovery of the 4 genes responsible for Lynch Syndrome. Informed by knowledge of the genetic basis of the disease, and propelled by the development and validation of 2 powerful clinical identifiers (MSI and IHC), clinicians currently have an extraordinary body of useful information about Lynch Syndrome, which has permitted progress in the diagnosis and treatment of this disease. There is still far to go in our understanding of Lynch Syndrome and the management of patients with this disease, but the past 20 years would lead one to be very encouraged about what the next 20 will bring.

Acknowledgments Supported by a grant from the NIH/NCI, R01 CA72851-17.

Conflict of interest The authors have no conflicts of interest to report.

References

1. Warthin AS (1913) Heredity with reference to carcinoma as shown by the study of the cases examined in the Pathological Laboratory of the University of Michigan, 1895–1912. Arch Int Med 12:546–555
2. Warthin AS (1925) The further study of a cancer family. J Cancer Res 9:279–286
3. Hauser IJ, Weller CV (1936) A further report on the cancer family of Warthin. Am J Cancer 27:434–449
4. Bargen JA, Mayo CW, Giffin LA (1941) Familial trends in human cancer. J Heredity 32:7
5. Savage D (1956) A family history of uterine and gastro-intestinal cancer. Br Med J 2(4988):341–343
6. Aure JC, Nilsson S (1964) Familial disposition of cancer of the gastrointestinal tract. Acta Chir Scand 129:644–648
7. Bieler VV, Heim U (1965) Double cancer in siblings. Familial association of cancer of the genitaliia and intestines. Schweiz Med Wochenschr 95:496–497
8. Glidzic V, Petrovic G (1968) Hereditary nature of cancers of the colon. Bull Cancer 55(4):511–516
9. Kartagener M, Wyler J (1966) Familial incidence of double malignancies. Schweiz Med Wochenschr 96(7):218–219
10. Kluge T (1964) Familial cancer of the colon. Acta Chir Scand 127:392–398
11. Heinzelmann F (1964) On a cancer family. A contribution to the problem of the hereditary aspects of colonic carcinoma. Helv Chir Acta 31:316–324
12. Lynch HT, Shaw MW, Magnuson CW, Larsen AL, Krush AJ (1966) Hereditary factors in cancer. Study of two large midwestern kindreds. Arch Intern Med 117(2):206–212
13. Lynch HT, Krush AJ (1967) Heredity and adenocarcinoma of the colon. Gastroenterology 53(4):517–527
14. Lynch HT, Krush AJ, Larsen AL (1967) Heredity and multiple primary malignant neoplasms: six cancer families. Am J Med Sci 254(3):322–329
15. Lynch HT, Krush AJ (1971) Cancer family "G" revisited: 1895–1970. Cancer 27(6):1505–1511
16. Douglas JA, Gruber SB, Meister KA et al (2005) History and molecular genetics of Lynch syndrome in family G: a century later. JAMA 294(17):2195–2202
17. Boland CR (1978) Cancer family syndrome. A case report and literature review. Am J Dig Dis 23(5):25s–27s
18. Boland CR, Troncale FJ (1984) Familial colonic cancer without antecedent polyposis. Ann Intern Med 100(5):700–701
19. Lynch HT, Riegert-Johnson DL, Snyder C et al (2011) Lynch syndrome-associated extracolonic tumors are rare in two extended families with the same EPCAM deletion. Am J Gastroenterol 106(10):1829–1836
20. Lynch HT, Kimberling W, Albano WA et al (1985) Hereditary nonpolyposis colorectal cancer (Lynch syndromes I and II). I. Clinical description of resource. Cancer 56(4):934–938
21. Lynch HT, Schuelke GS, Kimberling WJ et al (1985) Hereditary nonpolyposis colorectal cancer (Lynch syndromes I and II). II. Biomarker studies. Cancer 56(4):939–951
22. Boland CR (2005) Evolution of the nomenclature for the hereditary colorectal cancer syndromes. Fam Cancer 4(3):211–218
23. Lynch HT, Cristofaro G, Rozen P et al (2003) History of the international collaborative group on hereditary non polyposis colorectal cancer. Fam Cancer 2(suppl 1):3–5

24. Vasen HF, Mecklin JP, Khan PM, Lynch HT (1991) The international collaborative group on hereditary non-polyposis colorectal cancer (ICG-HNPCC). Dis Colon Rectum 34(5):424–425
25. Vasen HF, Watson P, Mecklin JP, Lynch HT (1999) New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the international collaborative group on HNPCC. Gastroenterology 116(6):1453–1456
26. Marra G, Boland CR (1995) Hereditary nonpolyposis colorectal cancer: the syndrome, the genes, and historical perspectives. J Natl Cancer Inst 87(15):1114–1125
27. Neale K, Bulow S (2003) Origins of the leeds castle polyposis group. Fam Cancer 2(suppl 1):1–2
28. Peinado MA, Malkhosyan S, Velazquez A, Perucho M (1992) Isolation and characterization of allelic losses and gains in colorectal tumors by arbitrarily primed polymerase chain reaction. Proc Natl Acad Sci USA 89(21):10065–10069
29. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M (1993) Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature 363(6429):558–561
30. Thibodeau SN, Bren G, Schaid D (1993) Microsatellite instability in cancer of the proximal colon. Science 260(5109):816–819
31. Peltomaki P, Aaltonen LA, Sistonen P et al (1993) Genetic mapping of a locus predisposing to human colorectal cancer. Science 260(5109):810–812
32. Aaltonen LA, Peltomaki P, Leach FS et al (1993) Clues to the pathogenesis of familial colorectal cancer. Science 260(5109):812–816
33. Fishel R, Lescoe MK, Rao MR et al (1993) The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 75(5):1027–1038
34. Leach FS, Nicolaides NC, Papadopoulos N et al (1993) Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 75(6):1215–1225
35. Parsons R, Li GM, Longley MJ et al (1993) Hypermutability and mismatch repair deficiency in RER + tumor cells. Cell 75(6): 1227–1236
36. Lindblom A, Tannergard P, Werelius B, Nordenskjold M (1993) Genetic mapping of a second locus predisposing to hereditary non-polyposis colon cancer. Nat Genet 5(3):279–282
37. Bronner CE, Baker SM, Morrison PT et al (1994) Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature 368(6468):258–261
38. Papadopoulos N, Nicolaides NC, Wei YF et al (1994) Mutation of a mutL homolog in hereditary colon cancer. Science 263 (5153):1625–1629
39. Nicolaides NC, Papadopoulos N, Liu B et al (1994) Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 371(6492):75–80
40. Palombo F, Gallinari P, Iaccarino I et al (1995) GTBP, a 160-kilodalton protein essential for mismatch-binding activity in human cells. Science 268(5219):1912–1914
41. Papadopoulos N, Nicolaides NC, Liu B et al (1995) Mutations of GTBP in genetically unstable cells. Science 268(5219):1915–1917
42. Miyaki M, Konishi M, Tanaka K et al (1997) Germline mutation of MSH6 as the cause of hereditary nonpolyposis colorectal cancer. Nat Genet 17(3):271–272
43. Boland CR, Thibodeau SN, Hamilton SR et al (1998) A national cancer institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 58(22):5248–5257
44. Umar A, Boland CR, Terdiman JP et al (2004) Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 96(4):261–268
45. Lindor NM, Rabe K, Petersen GM et al (2005) Lower cancer incidence in Amsterdam-I criteria families without mismatch repair deficiency: familial colorectal cancer type X. JAMA 293(16):1979–1985
46. Boland CR, Shike M (2010) Report from the Jerusalem workshop on Lynch syndrome-hereditary nonpolyposis colorectal cancer. Gastroenterology 138(7):2197
47. Goel A, Xicola RM, Nguyen TP et al (2010) Aberrant DNA methylation in hereditary nonpolyposis colorectal cancer without mismatch repair deficiency. Gastroenterology 138(5):1854–1862
48. Palomaki GE, McClain MR, Melillo S, Hampel HL, Thibodeau SN (2009) EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. Genet Med 11(1):42–65
49. Wijnen J, van der Klift H, Vasen H et al (1998) MSH2 genomic deletions are a frequent cause of HNPCC. Nat Genet 20(4):326–328
50. Yan H, Papadopoulos N, Marra G et al (2000) Conversion of diploidy to haploidy. Nature 403(6771):723–724
51. Ligtenberg MJ, Kuiper RP, Chan TL et al (2009) Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1. Nat Genet 41(1):112–117
52. Koi M, Umar A, Chauhan DP et al (1994) Human chromosome 3 corrects mismatch repair deficiency and microsatellite instability and reduces N-methyl-N'-nitro-N-nitrosoguanidine tolerance in colon tumor cells with homozygous hMLH1 mutation. Cancer Res 54(16):4308–4312
53. Hawn MT, Umar A, Carethers JM et al (1995) Evidence for a connection between the mismatch repair system and the G2 cell cycle checkpoint. Cancer Res 55(17):3721–3725
54. Carethers JM, Hawn MT, Chauhan DP et al (1996) Competency in mismatch repair prohibits clonal expansion of cancer cells treated with N-methyl-N'-nitro-N-nitrosoguanidine. J Clin Invest 98(1):199–206
55. Carethers JM, Chauhan DP, Fink D et al (1999) Mismatch repair proficiency and in vitro response to 5-fluorouracil. Gastroenterology 117(1):123–131
56. Aebi S, Kurdi-Haidar B, Gordon R et al (1996) Loss of DNA mismatch repair in acquired resistance to cisplatin. Cancer Res 56(13):3087–3090
57. Fink D, Nebel S, Aebi S et al (1996) The role of DNA mismatch repair in platinum drug resistance. Cancer Res 56(21):4881–4886
58. Arnold CN, Goel A, Boland CR (2003) Role of hMLH1 promoter hypermethylation in drug resistance to 5-fluorouracil in colorectal cancer cell lines. Int J Cancer 106(1):66–73
59. Elsaleh H, Joseph D, Grieu F, Zeps N, Spry N, Iacopetta B (2000) Association of tumour site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. Lancet 355(9217): 1745–1750
60. Boland CR, Goel A (2010) Microsatellite instability in colorectal cancer. Gastroenterology 138(6):2073–2087
61. Ribic CM, Sargent DJ, Moore MJ et al (2003) Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 349(3):247–257
62. Galon J, Costes A, Sanchez-Cabo F et al (2006) Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 313(5795):1960–1964
63. Fridman WH, Pages F, Sautes-Fridman C, Galon J (2012) The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 12(4):298–306
64. Burn J, Bishop DT, Mecklin JP et al (2008) Effect of aspirin or resistant starch on colorectal neoplasia in the Lynch syndrome. N Engl J Med 359(24):2567–2578
65. Burn J, Gerdes AM, Macrae F et al (2011) Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. Lancet 378(9809):2081–2087