HISTORY OF HEREDITARY NONPOLYPOSIS COLORECTAL CANCER OR "LYNCH SYNDROME"

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RESUMEN

Hereditary Nonpolyposis Colorectal Cancer (HNPCC or "Lynch syndrome"), involving pathogenic variants in the Mismatch Repair (MMR) genes, is the most common inherited condition that predisposed to colorectal adenomas and colorectal cancer. In this chapter I review the history of HNPCC, from early case reports, to more systematic clinical information and clinical criteria, and finally to the discovery of the MMR genes themselves in 1993, including the key feature of microsatellite instability. This central role for microsatellite analysis of colorectal cancers involves a growing trend toward "universal testing" for evidence of MSI, whether by PCR methods or by immunohistochemistry (IHC). Even though such universal testing has not been completely adopted around the world, we are already evolving toward more routine use of multi-gene "panels" for germline MMR mutation detection. Under these circumstances, one may reasonably ask whether understanding the historical unfolding of clinical features of HNPCC is even relevant. As this chapter hopes to demonstrate, an appreciation of the landscape of HNPCC would not be complete without such a historical perspective, including the important role of Dr Henry Lynch over a lifetime of work in the field.

Key words: Hereditary Nonpolyposis Colorectal Cancer (HNPCC), Lynch Syndrome, Mismatch Repair (MMR) Genes, Cancer genetics, Colorectal Cancer.

INTRODUCTION

As of 2017, a host of genes have been characterized, pathologic variants in which have been found to account for a large proportion of family history positive cases of colorectal cancer. They have been found to account for the occurrence of multiple polyps: adenomatous polyposis (APC and recessively acting MYH), hamartomatous polyposis (STK11 in Peutz-Jegher syndrome, SMAD 4 and BMPR1A in juvenile polyposis, PTEN in Cowden syndrome). More commonly, what appears to be inherited colorectal cancer risk does not involve multiple polyps, and for these cases we have hereditary nonpolyposis colorectal cancer (HNPCC or "Lynch syndrome") caused by variants in the mismatch repair or MMR genes, *MSH2*, *MLH1*, *MSH6*, and *PMS2*.

As molecular techniques have become more robust, additional genes with greater or lesser penetrance have been found to account for a smaller proportion of cases with and without multiple polyps. For those new to the field, much is to be learned about the clinical features of the various syndromes. Armed with even a superficial appreciation for the techniques of molecular testing, it is becoming increasingly easy to find an underlying inherited genetic basis for cancer in a given case. Once this has been achieved, management of an index case can be optimized and risk to relatives can be assigned, with opportunities for predictive testing in such relatives.

It is thus possible to practice clinical medicine and perform genetic counseling/ testing without a real appreciation of the historical development of the field of clinical cancer genetics.

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Nevertheless, those who wish a more thorough grounding, or are merely curious, may wish to read on in this chapter as I undertake to provide a historical perspective on the evolution of thinking about predisposition to gastrointestinal tract cancer, along with the stories behind emerging genetic technology.

In the broadest sense, the history of HNPCC falls into two periods, separated by the discovery of the mismatch repair genes and the epigenetic signature of microsatellite instability in 1993. Prior to this time, virtually all of the work was descriptive, involving efforts to distinguish a given family from the cluttered background of "chance aggregation", through the development of clinical features and criteria such as the Amsterdam criteria. Following discovery of the MMR genes, it was possible to achieve certainty in diagnosis for a given cancer patient and their family. Furthermore, with the newly-found ability to pinpoint carrier status in healthy at-risk relatives, much more aggressive and tailored surveillance was possible. In general, the events in the history of HNPCC have paralleled those encountered in the study and management of such conditions as hereditary breast & ovarian cancer (BRCA genes).

This chapter will very briefly summarize the terms that have been used to describe these disorders, including evolution in terminology, in the context of the temporal growth in understanding of clinical features. As genetic technology emerged, some reclassification in terminology has become necessary, and will be put in proper perspective.

Historical backdrop

The editor of Revista Médica Clínica Las Condes had originally invited Dr Henry Lynch to write this chapter, as he played a pivotal role in describing the clinical features of nonpolyposis inherited colorectal cancer. But due to infirmity, he has been unable to do so, and I have been asked to do so in his place. Since Henry Lynch is my father and since I began my own journey in the field of inherited GI cancer in his research department in the early 1970's, I am reasonably well-prepared to undertake this assignment. Before discussing HNPCC, a brief digression into Familial Adenomatous Polyposis (FAP) is more or less essential to a proper historical understanding.

Familial Polyposis

Before 1900, the association between multiple adenomas of the colon and colon cancer risk became apparent (Cripps, W, 1882). In this same era, the existence of an inherited susceptibility was recognized. It is important to appreciate that these observations came only a short time after Gregor Mendel's controversial experiments confirmed that autosomal dominant inheritance was a scientific fact, one that could enable prediction of risk to children and siblings of affected patients. In the 1800's pathologists were only beginning to understand that adenocarcinomas essentially always arose from dysplastic epithelium, typically the prosaic adenomatous polyp. That patients with hundreds to thousands of such adenomas were at particular risk of cancer was one of the key elements that hastened the appreciation of the adenoma-carcinoma sequence(Bussey 1987, Bulow, Berk et al. 2006).

This one rare condition, Familial (because it ran in families), Adenomatous (because the polyps were adenomas) and Polyposis (because there were lots of them), thus contributed to at least two key concepts: the potential heritability of cancer risk and the centrality of the adenoma to carcinoma pathway.

So FAP, by having an apparently consistent age-dependent progression, provided an ideal framework for developing prevention strategies. Prior to the routine use of modern flexible endoscopes, children of affected parents could be identified as having polyps on the basis of rigid proctoscopy and barium enema. When polyps were evident, prophylactic colectomy could be offered as a cancer risk-reducing strategy(Lockhart-Mummery, Dukes et al. 1956). Patients whose risk was so reduced, were then able to survive long enough to begin experiencing other consequences of the underlying susceptibility. The remaining rectum was at risk of cancer and, unless recurring polyps were successfully managed proctoscopically, the result was either rectal cancer or the need for further prophylaxis: completion proctectomy with end-ileostomy. This in turn led to better selection of patients for proctocolectomy as the initial measure if rectal polyp burden was high, and later to the development of strategies for restorative proctocolectomy (the construction of a neorectum with a segment of ileum, anastomosed to the distalmost rectum) (Parks and Nicholls 1978).

In many of these same patients it became evident that adenomas and cancer risk were not limited to the colorectum. Cancers of the stomach and the periampullary duodenum developed in a small number of patients that had been "cured" by colectomy or proctocolectomy. This then led to the emergence of surveillance of the UGI tract, usually beginning at or shortly after the patient's colon resection. Details of the approaches to surveillance of the pre- and post-operative lower and upper GI tract are beyond the scope of this discussion, but excellent reviews are available(Vasen, Bulow et al. 1997, Vasen 2000, Vasen, Moslein et al. 2008).

FAP also served as an early example of the tendency for cancer susceptibility to involve risk of cancerous and noncancerous complications across multiple organ systems. Decades before the APC gene was identified, cancer risk involving the

central nervous system (so-called Turcot's syndrome) and the thyroid had been demonstrated. Relatively uncommon, yet characteristic nonmalignant complications were also a feature of FAP, were demonstrated through the recognition of soft-tissue (epidermal cysts), bony (osteomas) and dental (supernumerary teeth) abnormalities, the triad of features comprising the Gardner syndrome variant of FAP.

Neither adenomas nor the sometimes occurrence of Gardner syndrome features were reliably able to identify carriers of risk before age 10-15. The discovery of congenital hypertrophy of the retinal pigment epithelium (CHRPE), also termed pigmental ocular fundic lesions (POFL) did enable the early characterization of carrier status in many families. For a short time before discovery and clinical use of APC, ophthalmologic screening had a place.

FAP registries

Most now take for granted the notion of "centers of excellence" for the expert management of both common and rare diseases. But this was not always the case. Specialists have been around for a long time, but not so much the specialty center. One of the very earliest programs of any kind for the registration and management of disease is the St Mark's hospital in London. It was originally founded as a specialty center for such benign colorectal diseases as anorectal fistulas and anal fissures. Under the leadership of the surgeon Cuthbert Dukes, well-known for the Dukes staging system for colorectal cancer, St Marks became a referral center for the management of FAP. Many of the leading GI surgeons, pathologists, and gastroenterologists from around the world have done a tour at St Marks. Although I never did train at St Marks, admission of personal bias requires that I mention a partnership (chemoprevention trial collaboration) and friendship of more than 20 years with Robin Phillips and several of his students. Building upon the approach taken by this single institution, a number of nation-wide and thus more truly population-based registries developed during the second half of the 20th century. Programs in Denmark, Finland, the Netherlands, Australia, New Zealand, Japan and other countries have all made important contributions in their own ways. In the US, a few institution-based registries emerged at about the same time as the programs in Europe and elsewhere. As with St Marks and the other international programs, these have, generally been an outgrowth of interest on the part of one or more colorectal surgeons.

FAP registries provided a basis for not only clinical management, but clinical research, including the advent of chemoprevention trials. Because FAP remains a rare disease, finding enough patients with an evaluable polyp burden required the utilization of services really only available at larger specialty centers. As we come to consider chemoprevention in HNPCC, it is necessary to pay a note of gratitude to those who undertook trials in FAP, establishing important precedents in trial organization and design. An obvious example is the series of CAPP trials in Europe. Many are familiar with the CAPP II trial which showed a cancer incidence and mortality benefit in HNPCC through the use of aspirin. What many may not fully appreciate is the fact that the network of participating centers and the administrative machinery had actually been established in the early CAPP I trial in FAP. It is hard to find a more direct link between an important HNPCC investigation and the antecedent work in FAP.

EVOLUTION OF HNPCC INVESTIGATION

Before exploring the history of HNPCC, a few words about terminology(Boland and Lynch 2013). The original term was "Cancer Family Syndrome" or CFS. Some have used the term Cancer Family Syndrome of Warthin or Cancer Family Syndrome of Lynch to honor Aldred Warthin, whose original 1913 paper set the stage for later work(Warthin 1913), or Henry Lynch, who, along with Anne Krush described many more of the nuances of the clinical picture of this condition. However, some confusion emerged as commentators referred also to the "Cancer Family Syndrome of Li and Fraumeni" now simply referred to as Li-Fraumeni syndrome (p53 germline mutations).

In order to avoid this confusion over "Cancer Family Syndrome" terminology and to provide a more descriptive name, the term Hereditary NonPolyposis Colon or Colorectal Cancer (HNPCC) was adopted in the course of some discussion by a series of working group meetings. The term was carefully thought out, if ultimately imperfect and insufficient. The term "hereditary" was considered essential to emphasize that, even though no gene had been identified, autosomal dominant inheritance patterns appeared again and again. "Nonpolyposis" was deemed essential in order to stress the absence of a significant number of polyps and to make it clear that this did not appear to be a variant of the more well-known and accepted FAP. "Colon cancer" was included as this was the most common tumor in virtually all seemingly affected families. Even as this term was widely gaining acceptance, it was being criticized for being too lengthy and for failing to emphasize that it was, in fact, characterized by a spectrum of extracolonic tumors.

For the reasons noted and to properly recognize the contributions of Henry Lynch, the term Lynch Syndrome was coined by Dr Rick Boland in an editorial(Boland and Troncale 1984, Boland 2005). This term stuck and is widely in use, though I prefer the older, more descriptive HNPCC, despite the noted limitations.

In order to more fully confuse and confound, several terminology modifications have emerged, with general though not universal acceptance.

HNPCC: Familial colon cancer or "FCC", not otherwise specified. Now commonly includes families meeting Amsterdam Criteria, whether or not microsatellite instability identified, but typically not referring to those in which a MMR mutation has been identified. When a MMR mutation is detected, the family is now more commonly referred to as having Lynch Syndrome.

Familial Colon Cancer "Syndrome X": Amsterdam Criteria for HNPCC met, but without MSI in tumors or underlying MMR mutation (Lindor, Rabe et al. 2005).

Lynch syndrome: MMR mutation found, whether Amsterdam Criteria met or not.

"Lynch-like": MSI present, not obviously somatically acquired, but no MMR mutation found (Boland 2013).

In light of the nuanced and confusing terminologies noted here, one is very tempted to invoke and adopt a term that recognizes the centrality of underlying MSI and of MMR, but none have really gained any acceptance. But it is quite likely that the evolution of terminology in HNPCC/Lynch Syndrome is not finished.

Early Reports

The contemporary sense of HNPCC dates from Warthin's 1913 report. However, there are likely many reports prior to this, simply less well documented. In a 1956 paper, Savage(Savage 1956) references a report of multiple cases of uterine cancer from 1857 (Paget). Reports from so long ago would have likely lacked good pathology documentation, and it is not certain how colorectal cancers would have been documented, with patient likely dying of bowel obstruction without antecedent diagnosis.

The first clear example of a family with HNPCC was that of "Family G" of Warthin, described in a case report in 1913. Many decades later this family was confirmed to have a MMR mutation. The original description included several generations of individuals, male and female, with colorectal and stomach cancer. Women were described as having cancers of the uterus. The pattern was consistent with autosomal dominance. The family was followed intermittently up to the 1960's. At about this time, Henry Lynch, a young medicine resident and medical oncology fellow, with prior graduate training in genetics, came upon an unrelated patient in Nebraska with a personal and family history of colon cancer. A literature review identified Family G to have generally similar features. He communicated with Dr Marjorie Shaw of the University of Michigan, who turned over archival records to Dr Lynch. He and his research associate, Anne Krush updated the information on Family G and reported the companion families(Lynch, Shaw et al. 1966, Lynch and Krush 1971). After years of productive work together, Anne Krush returned to her native Maryland where she managed the Johns Hopkins polyposis registry for the rest of her career.

During the early part of the 20th century, several other reports were describing potential heritability of colorectal cancer in more statistical terms, suggesting the existence of some degree of heritability. Small pedigrees of families that may or may not have had HNPCC were sometimes included, to show examples in which apparent heritability could be demonstrated.

During the 1970's, Henry Lynch was likely the first investigator to receive NIH grant support for family investigations, which were not limited to familial colorectal cancer. Case reports and more involved series of families with breast and ovarian cancer, melanoma, and others were published. At about this time, other clinical investigators from around the world were developing an interest in familial cancer syndromes, and would visit Dr Lynch for advice on how best to conduct such studies.

Additional clinical features of HNPCC were described during the 1970's and 1980's utilizing data from larger pools of families. These continued to rely on death certificates to document the presence of cancer or more helpfully, pathology reports from hospitals. Early databases enabled assembly of more clear association patterns. Among the features that emerged were a tendency toward involvement of the right colon(Lynch, Lynch et al. 1977), improved survival (Lynch 1975, Lynch, Bardawil et al. 1978), characteristic pathology (mucinous tumors and those with tumor-infiltrating lymphocytes), and expanding spectrum of tumors beyond the originally described colorectal and endometrial(Lynch and Lynch 1979, Fusaro, Lynch et al. 1980, Lynch, Lynch et al. 1981, Lynch, Smyrk et al. 1989).

Statistical Measures of Familiality

Although the repeated description of families around the world with similar features of early onset colorectal, endometrial, and other tumors, the absence of clearly distinguishing features, such as the diffuse polyposis of FAP, engendered considerable skepticism that any such condition as the "cancer family syndrome" truly existed or had an underlying genetic basis.

Several investigators took a different approach to the matter. While earlier approaches to measures of familiality do exist, the 1975 report by Lovett was really the first English language

report of a substantial case series. In her study, death certificates of sibs and parents of more than 200 colorectal cancer patients at St Marks were collected, with findings compared to the general population. Cases referred because of positive FH were excluded, but cases of rectal cancer were over-represented, in keeping with St Marks being a referral center for rectal cancer. The end-point of CRC mortality in relatives was relied upon instead of incidence, since death certification, including cause of death, had been regularly kept since 1930 in the UK. Medical records, which would have enabled documentation of nonfatal cancers, were not routinely a basis for reporting in the period leading up to this study. Correlation of reported cancer death with medical records was obtained when possible. Subject to whatever biases in reporting and computation may have existed, a significantly higher proportion of parents and sibs of colorectal cancer patients died of colorectal cancer than in the general population. Yet the proportions were not high enough to invoke simple inheritance as a basis for all or more cases. Lovett concluded: "More complex genetic mechanisms, involving heterogeneity, incomplete penetrance and multiple loci cannot be excluded"(Lovett 1976, Lovett 1976).

Somewhat later, in 1993, just before the report of the first likely HNPCC genetic locus, St John published a report from Australia, similar to that of Lovett, except that medical record documentation was obtained. A degree of familiality was described that was very similar to that reported by Lovett. In the St John report, risk to relatives was greater when an index case was younger than 55 at diagnosis or when an additional relative was affected with CRC.

COLLABORATIVE GROUPS: THE ICG-HNPCC

Through the 1980's, many investigators from around the world were recognizing and describing families with what looked like HNPCC. Additional clinical features were described and validated. As well, the development of population-based tumor registries enabled better estimates of heritability Momentum was gained when in 1989 Dr Paul Rozen hosted a meeting of investigators interested in HNPCC. Up until this time the only real organized collaboration in inherited colon cancer susceptibility was that of the Leeds Castle Polyposis Group or LCPG. This group formed in about 1975 at a conference held in the UK to commemorate an anniversary of the St Marks hospital in London. As many surgeons in attendance had spent time at St Marks, it was considered an opportune time to convene essentially a workshop on the problem of desmoid tumors in FAP and to try to reach consensus on classification and management. It was recognized that an important element, going forward, was the need to develop some consistency in the data collected on FAP in general. This first meeting was considered such a success that a working group was formalized with the intent of holding a biennial meeting and to undertake collaborative research. The best history of the LCPG is that of Neale and Bulow (Neale, Bulow et al. 2003)

When investigators met in Jerusalem in the important 1989 workshop, it clearly represented a mixture of those with experience in the LCPG and others that had not been a part of this small, relatively closed and fraternal group. In any event, since the mission of LCPG was disease specific, namely to deal with FAP, the decision was made to inaugurate a similar but distinct working-group devoted to HNPCC research and practice. Thus, the International Collaborative Group (ICG) on HNPCC was born(Vasen, Mecklin et al. 1991). Unlike the LCPG, the ICG-HNPCC met annually. Both the LCPG and ICG-HNPCC meetings were wonderful opportunities to meet with colleagues from around the world, and a real fellowship and sense of kinship and shared international mission emerged. Most collaborations developed between individual institutions rather than formally in the name of LCPG or ICG-HNPCC. Notwithstanding this limitation, such collaborations were very much fostered by the bonds that formed between individuals, not merely at the scientific sessions but at the dinners and social outings. I certainly treasure the memories of these early meetings and the friendships that emerged from them. The group photos represented a "who's who" in the field of HNPCC.

One of the important early products of the ICG-HNPCC was the formulation of the Amsterdam Criteria for HNPC)C (3 or more cases of CRC, occurring over 2 or more generations with 1 or more diagnosed below age 50, and 0 evidence of FAP, an easy to remember "3-2-1-0" rule(Vasen, Watson et al. 1999). The 1990 meeting in Amsterdam was hosted by Hans Vasen, who in my opinion has done more than anyone else to advance the field of HNPCC. Since the MMR genes of HNPCC had not yet been discovered, one of the key reasons for establishing clinical criteria was to have as much certainty as possible about the clinical diagnosis in a given family, in order that a recommendation for screening colonoscopy could have as strong a foundation as possible, given its cost need for frequent performance. Strict criteria also lent themselves well to selecting families most likely to be informative in the search for a susceptibility locus, though at the time this was a somewhat secondary focus.

As the years went by, and the MMR genes were identified (below), there was inevitable evolution and maturation of the ICG-HNPCC. Increasingly, it became obvious that for FAP and HNPCC the essential research and practice issues had more in common than they did differences. At a more practical level, it was becoming obvious that the surgeons, oncologists,

endoscopists, pathologists, genetic counselors, and laboratory investigators who were leaders in FAP were also leaders in HNPCC. And they were coming to realize that the cost of international meetings on such a regular basis was becoming too much to sustain. So a series of negotiations between the Councils of the LCPG and ICG-HNPCC met to discuss the possibility of merger. It was decided that for several more years the ICG would meet annually and LCPG biannually, but that they would meet back-to-back at the time of LCPG meetings. Ultimately a formal merger took place and both the LCPG and ICG-HNPCC "furled their flags" and a new organization, the International Society for Gastrointestinal Hereditary Tumors (InSiGHT) was formed. It now meets every two years and rotates between Europe, the Americas and Australia.

Regional Groups America and Australia

Not all needs are met by international meetings. In the interest of fostering regional collaborations and studies, the Collaborative Group of the Americas (CGA) on Inherited Colorectal Cancer was formed in the 1990's. Although this group holds annual meetings in the US, its focus is more on issues surrounding genetic counseling and indeed many of its members are genetic counselors. Its annual meeting is generally held at the time of either the American Society of Human Genetics (ASHG) or the National Society of Genetic Counselors (NSGC).

A European counterpart to the CGA developed more recently, the Mallorca Group. It has been successful in organizing data collections and has published European guidelines on management in HNPCC and other conditions. The successes of the CGA and Mallorca group has encouraged the formation of regional in more developing parts of the world. A series of meetings in South America have led to the formation of registries and programs for management of HNPCC and FAP.

DISCOVERY OF THE MISMATCH REPAIR GENES

Progress in molecular genetics of inherited colorectal cancer susceptibility really began with discovery of the APC gene responsible for FAP. A patient with both developmental abnormalities and FAP underwent karyotype analysis and was found to have a deletion involving chromosome 5. This raised the possibility that the locus responsible for FAP might be in this region. Positional cloning efforts soon led to identification of the responsible locus and shortly thereafter sequencing revealed the structure and function of the APC gene. Its important role in carcinogenesis is evident in a host of other tumors.

Discovery of a locus responsible for HNPCC was not so easily accomplished as it had been in FAP. It soon became clear from linkage studies that HNPCC was not a variant of FAP as it was not linked to the APC locus. Nor was it linked to other known genes. A much broader approach was taken. Large, informative families were required and a more genome-wide search was required, akin to the approach used to identity the BRCA genes responsible for hereditary breast and ovarian cancer.

The breakthrough came in 1993. In the Helsinki laboratory of Dr Albert de la Chapelle, Drs Paivi Peltomaki and Lauri Aaltonen evaluated DNA samples for several large families from New Zealand and Newfoundland and established linkage to a locus on chromosome 2 (Peltomaki, Aaltonen et al. 1993). Due to rapidly improving molecular techniques, it took only a few months to establish the sequence of the responsible gene(Fishel, Lescoe et al. 1993). It is worth noting that the Finnish group detected linkage in overseas families, as Finland was later found to have a major founder mutation involving the *MLH1* gene (chromosome 3) that would not have made Finnish families informatively positive at this chromosome 2 locus. Indeed, once linkage on chromosome 2 was firmly established in these two pioneer families, it was not found in a number of additional families, presumably including such Finnish *MLH1* kindreds.

The same issue of Science that reported the first genetic locus for HNPCC included a companion article showing that nearly all HNPCC colorectal cancers showed evidence of microsatellite instability (MSI), termed replication error or "RER" phenotype at the time(Aaltonen, Peltomaki et al. 1993). For several years basic investigation of yeast species had demonstrated the complex interaction of mismatch repair genes in identifying and editing single nucleotide and larger DNA replication errors, followed by recognition that such replication errors also occurred in human colorectal cancers(Ionov, Peinado et al. 1993, Fishel and Wilson 1997). In addition, Aaltonen and colleagues evaluated a series of evidently sporadic cases of CRC and found that 13% of such cases showed a similar pattern of microsatellite instability(Aaltonen, Salovaara et al. 1998). Like HNPCC families they showed a proclivity, toward involvement of the right colon and toward diploidy. These features have, of course, been validated in many subsequent series.

Important clues to the wider relevance of microsatellite instability were strongly hinted at in this seminal paper. As noted above, a number of additional HNPCC or HNPCC-like families did not map to the D2S123 locus on chromosome 2. Yet tumors from such families tended to show the same high rate of MSI as did those that showed such linkage, suggesting a common thread existed among tumors in HNPCC families, and perhaps hinting at the likelihood of additional genes involving a similar carcinogenic pathway. This of course did prove to be the case.

Less than one year was required from the identification of the D2S123 region on Ch2 to which the several HNPCC

families were linked to the identification and sequencing of the responsible gene. Fishel and colleagues and Leach et al(Ionov, Peinado et al. 1993, Leach, Nicolaides et al. 1993), utilizing the highly conserved known sequence of a yeast mismatch repair gene, *MSH2*, were able to identify a human sequence showing a high degree of homology (hence the term h (for human) Mut S Homologue 2, and then to map it to the regions identified by Peltomaki. They were able to confirm the identify of this h*MSH2* gene as the one responsible for some cases of HNPCC by identifying pathogenic sequence variants in the gene in cancer-affected members of several small HNPCC. Other families did not show evidence of h*MSH2* mutations, consistent with the findings of Peltomaki.

With the rapid characterization of the *hMSH2* gene, it was quickly evident that families clearly appearing to have HNPCC did not map to this locus. Led by Dr Annika Lindblom in Sweden, further research utilizing RFLPs and microsatellite markers in 3 families not mapping to the *hMSH2* locus led, within a few months, to an additional locus at DS31029 on chromosome 3 (Lindblom, Tannergard et al. 1993). As with the earlier report by Peltomaki, not all evaluated families showed linkage to this locus, suggesting the presence of still more mismatch repair genes in HNPCC. The gene residing at this locus was even more quickly identified the *hMSH2* a year earlier(Bronner, Baker et al. 1994, Papadopoulos, Nicolaides et al. 1994)

Additional mismatch repair genes soon followed, the *MSH6* and *PMS2* genes (Nicolaides, Papadopoulos et al. 1994).
Miyaki et al claimed to be the first to identify pathogenic mutations in the *MSH6* gene in 1997.(Miyaki, Konishi et al. 1997). In this first *MSH6* family, later onset and a high rate of endometrial cancer were noted, and such features are now of course known to be characteristic of *MSH6* families.

Microsatellite instability in colorectal tumors was found to be characteristic and essentially universal in HNPCC. However, when evaluating larger panels of colorectal tumors, it became evident that as many as 15% of all colorectal cancers displayed MSI(Liu, Nicolaides et al. 1995) (Thibodeau, French et al. 1998). Those not occurring in HNPCC-like families tended not to have germline mutations. They also tended to occur in older, female patients. Inactivation of the *MLH1* gene by hypermethylation of its promoter was found to be the typical basis for this sporadic MSI(Cunningham, Christensen et al. 1998). Performance of PCR-based assays generally requires a source of normal DNA to serve as a frame of reference against which to compare the increase or decrease in repeat sequences that defined the MSI tumor phenotype.

Due to the challenges in doing MSI testing, immunohistochemical testing was developed using labelled antibodies against MMR proteins. IHC carries the advantage of using tissue sections that requires no microdissection and, since it is based on loss of expression of mismatch repair gene-associated protein, the loss of protein expression in tumor but not normal mucosa points to the MMR gene that was likely mutated (Marcus, Madlensky et al. 1999, Lindor, Burgart et al. 2002). IHC has never been a perfect surrogate for MSI testing. Variable staining for *MSH6* protein has been observed in patients with *MSH6* mutations, and their tumors commonly show MSI at fewer loci (so-called MSI-low) than in patients with *MSH2* and *MLH1* mutations(Parc, Halling et al. 2000)

Bethesda guidelines for MSI testing

Due to the cost and logistical challenges of performing MSI testing (whether via PCR-based methods or using IHC) as well as germline mutation testing, patients with colorectal cancer had to be selected according to some clinical guidelines. It soon became evidence that requiring the presence of Amsterdam Criteria was too strict and would miss many patients with underlying MMR mutations. A consensus development conference was held at the NIH in Bethesda, Maryland, hence the Bethesda guidelines. These guidelines subsequently underwent revision (Rodriguez-Bigas, Boland et al. 1997, Umar, Boland et al. 2004)

Role of Population Studies

Even as clinical testing to identify MMR mutation carriers were being developed, questions regarding the frequency of HNPCC emerged. In an early study Aaltonen characterized a large series of CRC from Finland according to their MSI status(Aaltonen, Salovaara et al. 1998). An early estimate of MMR mutation frequency at the population was made, but various short-cuts in the testing process precluded any final conclusions about real frequency of MMR mutations in CRC.
More recent studies have gradually been able to use improvements in the techniques for tumor screening, such as utilizing both PCR-based MSI and IHC(Pinol, Castells et al. 2005), and evaluating for potential alterations in *MSH6* and PMS2(Hampel, Frankel et al. 2005), in addition to the "traditional" *MSH2* and *MLH1*. In the 2005 Pinol study from Spain, a large series of unselected cases of CRC were evaluated. Interestingly, the study was couched in terms of evaluating the accuracy of Bethesda guidelines. It was concluded that the Bethesda guidelines were relatively sensitive as a screening test for MMR carriage.(Pinol, Castells et al. 2005).

The later but also seminal Ohio series reported by Hampel at al has contributed to our understanding of the true frequency of MMR mutations among CRC patients. In that study, all 4 MMR genes were assessed by IHC and methylation assay was

performed to identify likely sporadic cases of MSI. In all 2.2% of cases were found to have underlying MMR mutations. It was conceded that, due to cost considerations, germline testing could not be done on all cases (ie, those that had normal IHC) That several cases identified by means of IHC abnormalities had not otherwise met Bethesda guidelines has likely contributed toward current practice guidelines in the US that provide for universal tumor testing (IHC on all CRCs) or near-universal (IHC on all below age 70 and those over 70 with a family history). (Giardiello, Allen et al. 2014, Provenzale, Gupta et al. 2016).

Since this chapter is about the history of HNPCC, the series of population studies, from Aaltonen to Pinol, to Hampel, mark an interesting evolution in the approach to characterizing incident case of CRC. This evolution has been based on increase in the number of relevant genes, simpler means for performing tumor testing, decreased cost of all such testing, and not least, willingness of patients, providers, and ethics boards alike to conduct such studies. All of these factors likely contribute to the noted liberalization of clinical practice guidelines for tumor testing.

Advent of "panel testing" and the use of a *priori risk*-assessment models

Although testing of tumors for evidence of MSI, mainly with IHC against MMR proteins, has become a standard of care for screening potential patients for underlying MMR germline susceptibility, there are other approaches that have recently begun to emerge.

Not all patients interested in having MMR germline testing have an evaluable tumor for testing. In other cases the "index patient" is deceased and it is an unaffected first-degree relative who wishes to have testing performed. In response to these situations, several a *priori risk*-assessment models have been developed, very similar to the models that have been used to determine whether a woman with breast cancer would benefit from BRCA testing.(Balaguer, Balmana et al. 2008, Kastrinos, Steyerberg et al. 2013). Some of these models include can include tumor testing while others do not. One cost-effectiveness study suggested that a prior probability of 5% or greater for carrying a MMR germline mutation was high enough to warrant testing.(Dinh, Rosner et al. 2011)

If a risk-assessment model can be used to screen patients that would benefit from mutational testing in the absence of MSI data from tumor, what has such mutational testing consisted of? In the US, most testing is done by a small number of competing reference laboratories. Initially these consisted of a narrow range of comprehensive tests for mutations in *MLH1, MSH2, MSH6, PMS2*, and *EPCAM*, performed by means of sequencing, coupled with testing for large rearrangements. These have proven generally satisfactory. However, given the high variability in reported personal and family cancer history and absence of tumor in many cases, the possibility of germline mutations in genes other than the MMR genes has become apparent. Just as testing for breast cancer susceptibility has extended beyond BRCA, to include PTEN, p53, and many others now comprising "panels" of potential breast cancer genes, a similar approach is emerging for colon cancer susceptibility. These do go beyond the scope of HNPCC as such, and are rapidly becoming adopted in US clinical practice. "Colorectal panels" include, in addition to the MMR genes and EPCAM, such genes as APC, MYH, SMAD4, STK11, BRCA, p53, ATM and many others that are not remotely regarded as HNPCC genes. This development has been due in part to the dramatic reduction in the cost of sequencing and because it requires less expertise to administer. Interpretation of such testing, involving the frequent detection of "variants of uncertain significance" or pathogenic variants in moderate penetrance genes, involves a whole host of issues that are beyond the scope of this discussion.

If use of panels is an emerging alternative or supplement to the use of tumor testing, what is the potential scope for such testing? Just as clinical selection criteria for tumor testing evolved over time, the threshold for use of panel testing has come under scrutiny. As noted above, a 5% prior probability has been proposed. Data have started to emerge on the potential use of panels testing in less and less "selected" cases. In one important recent study, Yergulen and colleagues in Boston, in cooperation with a large US reference laboratory, performed panel testing on all patients referred for possible HNPCC testing.(Yurgelun, Allen et al. 2015). Pehaps not surprisingly, a modest but arguably clinically relevant proportion of patients were found to have actionable pathogenic variants in non-MMR genes, including some with BRCA mutations. More recently, these same authors have undertaken to perform panel testing, universally, on an otherwise essentially unselected series of CRC cases from one academic institution. As expected, about 3% were found to carry MMR mutations. But an additional 6% were found to have some germline alteration in a non-MMR gene, including 1% with BRCA mutations. Tumor testing had predicted the MMR germline mutations, but would not have predicted the varios non-MMR mutations(Yurgelun, Kulke et al. 2017).

HNPCC TODAY

In 2017, there are well-accepted approaches to the identification and management of HNPCC. These approaches are embodied in several clinical practice guidelines(Balmana, Balaguer et al. 2013, Stoffel, Mangu et al. 2015, Syngal, Brand

et al. 2015, Provenzale, Gupta et al. 2016). Essentially these propose that all colorectal cancers be considered for MSI/IHC testing, and that when such testing is abnormal (excepting cases in which *MLH1* hypermethylation or BRAF mutation is identified), performance of germline MMR testing be done. A host of variation on this theme can be considered, including more selective application of MSI/IHC by limiting testing to those under age 70 or by imposing various other clinical requirements. The recent introduction of relatively low-cost "panel testing" for germline alterations in not only MMR genes but a variety of other genes not involving underlying MSI may well revolutionize the approach to testing.

Once a MMR mutation is identified, important clinical screening and management issues need to be addressed. It is almost universally recommended that mutation carriers, with or without prior CRC, undergo colonoscopy for early detection of cancer or adenomas beginning by age 20-25, due to high risk of early onset, and be repeated at 1-3 year intervals, due to rapid growth and transformation of adenomas. Even in this most well-accepted screening, controversies abound: should those with *MSH6* and *PMS2* mutations have initiation delayed until age 30 or 35 due to lower penetrance? Given a rate of interval cancers that is unacceptable to some, should enhanced imaging techniques be employed (narrow band imaging, chromoendoscopy)? Should supplemental methods, such as assay for mutated DNA in the stool, be considered? In light of the favorable prognosis associated with colonoscopy-detected CRC, should we be less obsessive with colonoscopy surveillance and accept that a small proportion of patients will need CRC surgery for cancers that are missed despite aggressive screening? This conclusion was hinted at in the recent, important studies by Moller (Moller, Seppala et al. 2016, Moller, Seppala et al. 2017) and colleagues from many European countries, in which a large number of MMR mutation carriers under active colonoscopy screening were found to have an unsettlingly high number of colorectal cancers, despite this surveillance. Reassuringly, the long-term survival in these patients was well over 90%.

With respect to risk of extracolonic tumors, less evidence exists in support of recommendation for screening and no well-conducted studies have been performed. Many of us perform UGI endoscopy at the time of colonoscopy or, more commonly, with alternating colonoscopies, as it is easy to do. Those with an immediate FH of UGI cancer, personal history of H pylori infection, or residence in a high-risk geography for stomach cancer, may be targeted for such surveillance, but no data exist in support of such an approach.

Gynecologic management is controversial. Endometrial cancer risk is high, but most such cancers present with early symptoms and it carries a low mortality in general. Risk can be eliminated altogether with prophylactic hysterectomy(Schmeler, Lynch et al. 2006). Ovarian cancer has always been considered to carry a much more grave prognosis. Transvaginal ultrasound screening is widely employed, but sensitivity and specificity for early tumors are very poor. Fortunately, data are emerging suggesting that, as with so many other HNPCC tumors, the natural history of ovarian cancer may be much more favorable than traditionally thought. In any case, prophylactic oophorectomy is curative.

Rates of uroepithelial tumors are high, mainly in *MSH2* carriers. Many employ noninvasive measures such as urinalysis (for microscopic hematuria) or urine cytology. However, no such screening test has been properly validated.

Considerable controversy surrounds the question of whether prostate and/or breast cancer are part of the HNPCC spectrum at all. MSI has been demonstrated in these tumors from known MMR mutation carriers, but the rates for these tumor are only marginally increased over population expectations. Consequently, no recommendations for enhanced screening have been offered.

An important unmet need in nearly all HNPCC families involved cascade testing, namely the process for identifying at-risk relative of mutation carriers and for engaging them in genetic counseling, testing, and clinical screening. In the US and most Western countries the burden of such "outreach" falls on the index case himself or herself. A genetic counselor may provide this patient an excellent verbal and written summary of the issues in HNPCC testing and screening, and may stress the importance of conveying this to at-risk relatives. But if the index case bears the entire burden of reaching out to relatives, follow-through may fail for a host of reasons.

In several countries provider-mediated outreach has become the norm. This involves a very simple stepwise process:

1. Identify mutation carrier

2. Ask this carrier to complete a form containing known, at-risk relatives and their contact information

3. The provider corresponds directly with such identified relatives and provided information about the condition in question (HNPCC) and summarizes the risks and benefits of genetic testing and screening, and provides information about genetics and medical providers that can arrange for testing, screening, and management. These programs, available in South Australia(Suthers, Armstrong et al. 2006) and nationwide in New Zealand (Susan Parry, personal communication), are operated by government-operated health ministries, with the credibility that accompanies such services in these countries. We are exploring web-based strategies for carrying out

the same tasks. Challenges include development of suitable web-based software and the adoption of appropriate internet security safeguards, privacy and confidentiality assurances.

THE FUTURE OF HNPCC

The implications of multi-gene panel testing raise the possibility that we may soon be able to "test everybody for everything". Already in many countries newborn testing is mandatory and carried out for conditions much less common than HNPCC. The challenges do doing so involve important issues of cost, cost-effectiveness, and the willingness of individuals and society to accept such an undertaking. But it is important to recognize that it is already technically possible to do so.

Until such time as cancer susceptibility testing is routinely available to the worried-well, it is possible that state and national tumor registries will be able to at least give cancer-care providers feed-back regarding the extent to which they are meeting guidelines for referral of CRC cases for counseling and testing. This is already being piloted in several US states and there is existing commentary on the subject(Hollands, French et al. 2016, Khoury and Galea 2016)

When all survivors and relatives at-risk have their carrier status established, screening and prevention measures with become paramount. Less invasive and more sensitive measures for identifying precancerous adenomas are required and will likely be part of the near-future technology advances. Surveillance trials for extracolonic tumors in the HNPCC spectrum are overdue. I hope that collaborative groups such as InSiGHT and the regional groups will take up this challenge.

CONCLUSION

In the short 24 years since 1993 when discovery of HNPCC-associated genes was first reported, I could only have imagined the explosion of information about this condition and the ways in which genetic technology would empower patients and providers alike.

I lack the imagination to predict what the landscape will look like another 24 years on.

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