The evolution of colorectal cancer genetics—Part 1: from discovery to practice

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Abstract: Colorectal cancer (CRC) is an increasing burden on our society. Identifying those who are at the greatest risk and improving triage for treatment will have the greatest impact on healthcare. CRC is a prime paradigm for cancer genetics: the majority of disease results from stages of progression lending itself to prevention by early detection of the pre-disease (neoplastic) state. Approximately 10% represent well defined hereditary cancer syndromes. Hereditary CRC has the added benefit that many are slow growing and family members are armed with the knowledge of potential risk of associated carcinomas and empowerment to reduce the disease burden. This knowledge provides the indication for early endoscopic and/or surgical intervention for prevention or treatment of an entire family cohort. The molecular basis of CRC allows enhanced characterization of carcinomas, leading to targeted therapies.

Keywords: Colorectal cancer (CRC); genetics; prevention; CRC syndromes; Lynch syndrome (LS)

Introduction

The role of genetics in colorectal cancer (CRC) has become critical to the mission of disease prevention, early detection and effective treatment. Over the last century, CRC genetics has emerged from an unrecognized to a specialized field, encompassing all aspects of cancer care. CRC is a preventable disease. The natural history of CRC differs in individuals with a hereditary predisposition: with an abbreviated length of tumorigenesis, often presenting at an earlier age. The incorporation of cancer risk assessment (CRA) and presymptomatic genetic testing results in effective stratification. Identification of high-risk individuals/families leads to more appropriate screening, options of prophylactic surgery for primary prevention and knowledge of potential associated cancers. Moreover, given the autosomal dominant inheritance of most CRC syndromes, 50% of a family cohort will be spared increased surveillance and anxiety associated with a positive family history.

Background

As infectious diseases have waned, and healthcare has improved, we are faced with diseases that occur at ages not previously attained. CRC is the third most common cause of cancer death in the world and is estimated to have an incidence of over one million cases per year (1). Research has led to micro diagnosis and improved systemic treatment however, despite advances in detection and care, morbidity and mortality from CRC continues to be high. Incorporating medical genetics dramatically improves outcomes at the public health level.

This review is a tribute to the pioneers who possess the ingenuity, perseverance, and collaborative nature to painstakingly (often with no support) collect data across generations, and laboriously isolate and analyze DNA. Throughout the last 100 years, as their discoveries blossom, we are awarded with the fruits of their labor.

History

The sentinel account of a hereditary colorectal family was by Dr. Aldred Warthin, who first suspected the disorder in the family of an affected woman (who subsequently died of endometrial cancer) over 100 years ago. He began studying her family (Family G) in 1895 and published his first report on it in 1913 documenting a pattern of gynecological cancer—specifically endometrial cancer—and gastrointestinal cancers, particularly gastric and colon (2). In 1971, updated studies of Family G by Lynch and Krush showed it to be consonant with what became known as Lynch syndrome (LS) (3). A marked 70-80% percent excess of proximal colon cancers was observed in patients with LS (4). Cutaneous manifestations of the Muir-Torre syndrome, such as sebaceous adenomas and sebaceous carcinomas also were found to be associated with the disorder (5). CRCs are the most frequent cancers associated with LS; endometrial cancers have been identified as the second-leading cancer associated with the syndrome. The MutS, E. Coli, Homolog of, 2 mutation was subsequently identified in Family G in 2000 (6). With current detection and treatment options, it is felt that no one with LS should die of CRC, assuming that the patient at increased risk has been identified, has a knowledgeable physician, and has been referred to a gastroenterologist or surgeon who prescribes frequent (annual) screening colonoscopies initiated at age 25.

Knudson's two hit hypothesis provided the basis of our understanding of how tumor suppressor genes could explain the younger ages of onset in familial cancers as well as variable penetrance. Although susceptibility is increased, a second mutation is required to produce a tumor (7,8). Fearon & Vogelstein showed us that in some cancers, the adenomatous polyposis coli (APC) gene is mutated as the initial step in the carcinogenic pathway (9). Mutations in the adenomatous polyposis coli gene are responsible for the syndrome originally recognized in the 1930's as autosomal dominant familial severe polyposis, currently known as familial adenomatous polyposis (FAP) (10-12).

Once some of the putative genes for colon cancer were identified, the value of a detailed family history became apparent. The expanded histories often led to the characterization of hereditary cancer syndromes and a better understanding of the natural history. For the first time, phenotypes could be predicted from the genotype providing valuable information towards prevention. The locations of mutations in the APC gene were shown to be associated with extracolonic manifestations as well as the severity and age of onset of polyposis (13). Shortly thereafter the extracolonic cancers in LS were confirmed (14-16). Identification of the familial mutation allowed pre-symptomatic genetic testing of family members opening the possibility of prevention and early detection of related cancers. Equally important is the sparing of those who are mutation negative thus reducing the psychological ramifications of the unknown.

In 1990 Congress awarded $3 billion to the Human Genome Project (HGP) which was completed with an international consortium in 2003. The hopes of genomic information raised the possibility of unforeseen consequences. For example, the Ethical, Social and Legal Implications (ELSI) committee was established to deal with the non-technical impact of this knowledge. A new branch of the National Institutes of Health, the National Human Genome Research Institute (NHGRI) is the result of the HGP and dedicates 5% of the budget towards ELSI which continues to guide us through this exciting social transformation.

Technological advances provided a boost towards new genetic discoveries launching the arena for high throughput analysis. Large amounts of data are now available in a short amount of time with small amounts of DNA. Our understanding of CRC continues to grow, and it is now estimated that up to 10% of the population has a known hereditary CRC syndrome. More importantly, there are 20-30% of CRC cases with evidence of a familial component, but without an identified hereditary gene mutation (1,17,18). Genetics has increased our understanding of the somatic events of tumorigenesis. The molecular pathology of the tumor describes two pathways to carcinogenesis mismatch repair and serrated polyposis (19,20). More recently, we have come to appreciate how cancer can be caused by the epigenetic modification of cancer genes, both heritable and acquired.

Genetic counseling (cancer risk assessment, CRA)

Genetic counseling is the process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease. This process integrates: (I) interpretation of family and

medical histories to assess the chance of disease occurrence or recurrence; and (II) education about inheritance, testing, management, prevention, resources and research, and counseling to promote informed choices and adaptation to the risk or condition (21).

CRA is a specialized area of genetic counseling and is an integral component of cancer care and prevention in a modern healthcare system. CRA is the process of obtaining a family history, detailed medical and surgical history, psychosocial assessment, risk counseling, education regarding preventative measures, and natural history of disease, discussion of genetic testing and informed consent. Guidelines for offering CRA are documented with position statements by leading healthcare organizations such as The National Comprehensive Cancer Network (NCCN), American Society of Clinical Oncology (ASCO), American College of Gastroenterology, National Society of Genetic Counselors (NSGC) and Collaborative Group of the Americas on Inherited Colorectal Cancer (CGA-ICC) (22,23). The NCCN 2014 clinical practice guidelines provide guidance for the management of high-risk patients with a hereditary cancer predisposition. In addition: "all individuals with CRC should be considered for a risk assessment with collection of family history" (24). Screening and predisposition genetic testing has introduced new opportunities along with fear of developing disease. Due the complex nature of cancer genetic testing, pre and post-test genetic counseling is recommended by NCCN, ASCO, American College of Physicians, and American College of Medical Genetics.

Qureshi *et al.* note that family history is a fundamental component of health information and therein all primary care physicians should have as a core skill the ability to take an adequate and accurate family history, even though few questionnaires have been developed for, and evaluated in, primary care settings (25). Further, few questionnaires "… have been compared with either gold standard (genetic interview) or current primary care 'standard practice' (family history as recorded in charts)…". The limited evidence, which depends on extrapolation from studies in settings other than primary care, suggests that systematic questionnaires may significantly improve the family health information gathered under current primary care practice.

While the above is essential for data gathering in the quest for a presumptive diagnosis, patients at high risk will profit immensely by being evaluated by a knowledgeable physician, genetic counselor, and/or center of genetic expertise. Hampel *et al.* discuss decision making for cancer genetics consultation,

based in part on criteria from consensus statements such as those from the NCCN as well as other publications whenever guidelines have been defined (26). In the case of the LS, for example, they suggest any of the following as high risk: (I) three first-degree or second-degree relatives (SDRs) affected with any LS associated cancers, wherein all cases can occur in one generation with no age restriction; (II) one first-degree relative (FDR) or SDR with two or more LS associated cancers; (III) one FDR with CRC earlier than 50 years of age. They suggest the following as moderate risk: (I) one FDR with CRC diagnosed at age 50 or later and one SDR with CRC at any age; (II) two FDRs with CRC diagnosed at any age, including age 50 years and later. They concluded that these criteria should improve the ease of referral and add to the promotion of consistency among hereditary cancer specialty centers when evaluating patients for referral to such specialists.

The aim of Rubin *et al.* was to determine whether patients with CRC are aware of the risk to their family members and to investigate an educational intervention (27). Two hundred fifty-three CRC patients agreed to participate in the study, but only 120 (47.4%) were aware that their FDRs were at increased risk for CRC. An educational survey instrument was developed to assess patients' understanding of family risk of CRC, coupled with the importance of early surveillance, which served to educate them about CRC screening guidelines. An educational and assessment brochure was provided for patient reference as a targeted intervention. They were then contacted by telephone and requested to complete a similar survey. In primary analysis of its effectiveness, it was found that less than half acknowledged that they understood their increased risk when compared with general population expectations. In addition, 34.8% believed that their FDRs possessed the same risk of CRC as the general population. Of further interest was the finding that 14.2% believed that their FDRs were at lower risk than the general population. Among those patients who understood that their FDRs were at increased risk, "…91.7% reported that they have warned their family members about their increased CRC risk, but only 56.7% could state the correct recommended age for screening within five years".

Nearly half (45.8%) of all patients surveyed mentioned that their doctor was their source for knowledge about CRC risk with primary care physicians and gastroenterologists being the most commonly identified, followed by oncologists and surgeons. After doctors, magazines were identified as the second most likely source of information

regarding colon cancer risk (15.8%). Finally, with respect to the post-educational intervention, it was found that this did not increase patient understanding of familial CRC risk, even among those who reported reading it. This study is believed to be the first to evaluate the communication of CRC risk from a patient to an at-risk family member. Of particular significance was the finding that more than half of these patients were found to have an inadequate understanding of familial risk, coupled with the fact that a mailed educational intervention was unsuccessful in educating these patients. These findings stress that family information services, using direct patient contact, is more effective than mailed or telephoned pursuits. More research clearly is needed in this vital and potentially life-saving communication process, especially where it involves communication between family members.

Domanska *et al*. call attention to the need to identify and adequately manage patients at risk for LS, since this knowledge could be effectively translated into surveillance programs in the interest of reducing morbidity and mortality (28). These authors used a questionnaire that was answered by 67 mutation carriers and 102 physicians from a health care region in Switzerland. Both groups answered questions pertaining to CRC risk, surveillance, and genetic testing, but, unfortunately, answers about inheritance and risk for LS-associated cancers were less accurate. Unfortunately, only half of the family members and one-third of the physicians correctly estimated the risk to inherit a LS-predisposing mutation. These findings reflect the challenge to physicians in keeping up-to-date on hereditary cancer.

Wong *et al*. utilized an informatics program enabling them to link data from a prospective CRC database from four hospitals in Melbourne, Australia, wherein they were able to determine the number of patients who, on the basis of at least one risk factor for hereditary CRC, could then be considered for Familial Cancer Clinics (FCC) which enable counseling of patients and families about risk reduction strategies followed by genetic testing when appropriate (29). Their findings showed that "Of the 829 new diagnoses of CRC 228 (27.5%) would potentially have benefited from FCC referral. Of these, 50 persons (21.9%) were referred and 32 (14.0%) attended. The highest referral rates were in young, early-stage CRC patients with a family history and the lowest in late-stage and multiple-polyp patients. Patient sex, language and insurance status did not influence referral or attendance." These findings suggest that appropriate FCC referral is low and that "…certain subgroups are at particular risk of non-referral and that many referred

patients do not ultimately attend. Interventions that increase referral rates and encourage attendance need to be considered."

Sweet *et al*. compared the extent to which a detailed family history was present in the physician's medical record in the setting of a touch-screen family history computer program (30). The study comprised 362 patients who were evaluated at a comprehensive cancer center ambulatory clinic over a one-year period and who voluntarily used the computer program. The computer entry was then evaluated by genetic staff and compared with the medical record for corroboration of family history findings, followed by appropriate physician assessment. Family history findings from the medical record were identifiable for comparison to the computer entry in 69% of the 362 computer entries; only 101 were assigned to a high-risk category. Yet, evidence from the records was able to confirm only 69 high-risk individuals. Furthermore, "… Documentation of physician risk assessment (i.e., notation of significant family cancer history) was found in only 14 of the high-risk charts. Only seven high-risk individuals (6.9%) had evidence of referral for genetic consultation." These findings clearly demonstrate the necessity for, and failure, for the sufficiently detailed collection of family history on all new and established patients so that an adequate CRA can be achieved.

Tyler and Snyder reviewed ambulatory records of 734 patients relevant to CRA and characterized them as suggestive of average, moderate, or high genetic risk for cancer (31). Those patients with a family history of CRC, modification of CRC screening were assessed to reflect degree of cancer risk wherein the frequency of cancer genetic referral in such high-risk patients was noted. While the family history was documented in 97.8% of the medical records, there nevertheless were insufficient findings "… to adequately assess risk in 69.5% of the charts. Detail of family cancer documentation was associated with personal history of cancer (P<0.01), patient age (P<0.01), and physician training status (P=0.04), but not with patient or physician gender, duration of care, or completion of a pedigree. For persons with a family history of CRC, compliance with cancer screening individualized to degree of risk was achieved in 50% of patients. Ten patients met criteria for moderate or high genetic risk for cancer. None had been offered cancer genetics consultation." The authors concluded that, while all records documented the presence or absence of a family history of cancer, nevertheless, "…in those with a positive family history, the detail of information was insufficient to permit risk assessment in over two thirds of individuals; risk-stratified colon cancer screening was

not achieved in half of the patients with a positive family history of CRC; individuals at moderate or high cancer risk were not identified as such; and those at high risk were not offered cancer genetics referral…". Clearly, family physicians must adopt explicit risk assessment criteria to enable assessment criteria that could lead to optimal care for those patients at increased hereditary cancer risk.

Ait Ouakrim et al. note that patients with a family history of CRC may show a substantial benefit from most kinds of screening and therein such screening could be cost effective (32,33). Specifically, CRC screening guidelines are generally more aggressive among persons with an established cancer-prone family history when compared with those who are at general population risk (34). However, in reviewing the literature, these investigators found that there is only limited information that depicts the level of screening uptake coupled with screening practices and/or the level of adherence to recommended screening guidelines. They quote the work of Rees et al. who comprised a review of 14 studies on the screening participation of FDRs of persons with CRC, and therein findings disclosed that only a few investigations had specifically studied screening uptake among those at increased risk through family history (35). In addition, many of these investigations were unable to provide details of the family history sufficient enough to determine if the screening was based upon risk-appropriate recommended screening intervals. Ait Ouakrim et al. concluded that there was a paucity of information relevant to those factors which best influence screening behavior among individuals with a strong family history of CRC (32).

Given these limitations in knowledge about screening behavior, Ait Ouakrim et al. used a population-based family study approach in order to estimate the CRC screening practices among unaffected Australians who were at increased familial risk (32). This enabled them to examine the association between self-reported screening behavior and socio-demographic factors. Their study involved 1,236 participants at moderately increased risk of CRC, wherein 70 (6%) "…reported having undergone guideline-defined 'appropriate' screening, 251 (20%) reported some, but less than appropriate screening, and 915 (74%) reported never having had any CRC screening test. Of the 392 participants at potentially high risk of CRC, 3 (1%) reported appropriate screening, 140 (36%) reported some, but less than appropriate screening, and 249 (64%) reported never having had any CRC screening test…". Factors associated with compliance were patients of middle age who were more highly educated and who had resided in Australia for

a longer period of time. It was concluded that guidelines for CRC screening were simply not being implemented in the population and there is a dire need to implement more effective strategies for population screening.

Ait Ouakrim et al. report the first population-based study incorporating risk-category-specific estimates of CRC (32). The level of screening uptake was found to be low in both moderate and high-risk categories. Specifically, "…Of 1,236 participants considered at increased risk for CRC, only about one in four reported ever having a screening colonoscopy and only one in 15 screened according to published guidelines. Participation in colonoscopy screening was slight for participants at potentially high risk of CRC for whom one in three had some screening, but only about one in 130 had appropriate screening." The main strength of the Ait Ouakrim et al. study was their ability to examine screening participation in accordance with specific CRC risk levels as defined by family history of cancer. Attention was called to the findings of Dove-Edwin et al. who showed that screening is known to reduce CRC risk for persons with a positive family history (36). Furthermore, Ait Ouakrim et al. have shown that the majority of such persons undergo inappropriate screening or no screening at all, thereby demonstrating the loss of a potentially preventable CRC occurrence in their Australian population which, incidentally, has one of the highest incidence of CRC in the world, with more than 13,500 cases diagnosed each year and an adjusted incidence rate of 38.7 per 100,000 persons (32,37,38). Attention was called to the fact that "Medical practitioners are often not familiar with CRC screening guidelines or not proactive in implementing them (39). Given that patients' compliance with guidelines is unlikely without their doctor's influence and encouragement, we speculate that our findings remain relevant to the current Australian context, as no major or specific initiative to increase screening participation by people above average-risk of CRC has been implemented during the last decade…" (40,41).

Inherited colorectal cancer (CRC)

From a genetic perspective CRC can be grouped into three categories: sporadic (75% of cases), familial (20% of cases) and hereditary (10% of cases). Sporadic cases have no apparent indications of a hereditary component. Familial cases have a family history of CRC that suggests multifactorial hereditary factors or common exposures to non-genetic risk factors or both. Inherited highly penetrant single gene genetic mutations account for about

5% of cancer cases. This review focuses on the genetics of hereditary cancers particularly LS but also FAP and MUTYH-associated polyposis (MAP) (1).

Lynch syndrome (LS)

LS, also referred to as hereditary nonpolyposis colon cancer (HNPCC), is the most common autosomal dominant cancer predisposition syndrome responsible for about 3% of all cancer cases. Two variant forms of LS are recognized: Muir-Torre syndrome (LS and sebaceous adenomas) and Turcot syndrome (LS and glioblastoma). Patients with LS have an 80% lifetime risk of CRC and women have a 60% risk of endometrial cancer. In addition they have an elevated risk of other cancers including stomach, biliary, ovarian and urogenital cancers. Rare individuals who inherit biallelic mis-match repair gene mutations have severe disease often presenting in childhood with hematologic cancers, brain tumors and early onset colon cancer, a condition referred to as constitutional MMR deficiency syndrome (42,43).

LS has the following cardinal features (44,45):
- Early age of cancer onset;
- Proximal colon involvement of CRC;
- Increased incidence of synchronous and metachronous CRCs;
- Autosomal dominant inheritance pattern and MMR germline mutation, most common of which are *MSH2*, *MutL*, *E. Coli*, *Homolog of*, *1*, and *MutS*, *E. Coli*, *Homolog of*, *6*;
- An excess of extracolonic adenocarcinomas;
- Frequent occurrence of distinctive pathologic features;
- Increased survival from CRCs (33);
- Accelerated carcinogenesis and interval CRC.

The LS is characterized by a defect in the mis-match repair process. This is a specific type of DNA repair involving the identification and repair of mis-incorporation of bases, largely due to replication and recombination, but also some forms of DNA damage (46,47). This specific DNA repair defect offers a very exact screening method for inherited colon cancer, namely "microsatellite instability", or MSI. Testing involves comparing tumor and non-tumor tissue for changes in size of stretches of poly-nucleotides, which are particularly prone to the same type of insertion/deletion mutations that result from aberrant DNA replication. While all forms of LS include defects in mismatch repair the reverse is not true, as this repair pathway may be impaired through somatic (non-inherited) mutations, such as aberrant methylation, aberrant expression of other genes in the MMR

pathway, degradation of mRNA via targeted microRNA overexpression (48). Inherited mutations are detected by sequencing the entire set of genes: (I) MLH1 located on chromosome 3p21.3 accounts for 50% of cases; (II) MSH2 located on chromosome 2p22 accounts for 40% of cases; (III) MSH6 located on chromosome 2p16 accounts for 7% of cases and PMS2 located on chromosome 7p22 accounts for less than 5% of cases; (IV) epithelial cellular adhesion molecule gene (also called tumor-associated calcium signal transducer 1) located upstream of MSH2 on chromosome 2p21 accounts for 1-3% of cases and can lead to inherited epigenetic silencing of MSH2 (43,49,50). For this reason, it is suggested that sequencing also include a substantial portion of neighboring DNA. Somatic (i.e., non-inherited) inactivation of the MLH1 gene can occur by methylation and often results in an absence of this protein that can be detected through immunohistochemistry but is not as reliable a test when compared to MSI as the protein may be present but is non-functional. An abnormal MSI or IHC result is used to determine the appropriate next set of tests, either germline testing or BRAF and MLH1 promoter methylation analysis (43,51).

About 15% of sporadic CRCs will manifest MSI along with absent MLH1 and PMS2 expression on IHC. In sporadic tumors the loss of MLH1 results from methylation of the MLH1 promoter in the somatic cells of the tumor only and not in the patient's normal cells. The absent PMS2 expression results because PMS2 normally forms a stable complex with MLH1 and in the absence of MLH1 PMS2 is unstable and degraded. Also, over half of sporadic tumors with loss of MLH1 expression have a mutation in the BRAF gene (p.V600E), a mutation that is not found in patients with LS associated cancers (43,49,51).

For germline mutation analysis Sanger sequencing, as opposed to "next-generation" sequencing, of all coding exons and flanking intronic regions of all MMR genes is the gold standard for mutation detection. In the case of PMS2 the presence of multiple highly homologous pseudogenes is problematic and necessitates the need for locus-specific, long range PCR amplification. Special methods are used for detection of large gene rearrangements (such as deletions or duplications of entire exons) as these lie beyond the limits of sequencing technologies. Such rearrangements are not uncommon especially in the MSH2 gene. The multiplex ligation-dependent probe amplification (MLPA) test is often used to detect large rearrangements. If only one exon is deleted by MLPA analysis using a second confirmatory test (for example, real-time PCR) is often used to confirm

the result. Comparative genomic hybridization (CGH) with gene-targeted arrays can also be used for detection of exon deletions or duplications (43,49,51).

A deletion of the 3'-end of the EpCAM gene located upstream of MSH2 causes transcriptional read through from the EpCAM gene and resultant silencing of the MSH2 gene by promoter methylation. EpCAM 3' exon deletions are readily detected by MLPA analysis. EpCAM deletion carriers have a risk of CRC similar to that of MSH2 mutation carriers. The risk of endometrial cancer in female EpCAM carriers is lower but if the deletion extends close to the MSH2 gene the risk of endometrial is much increased. The EpCAM gene encodes the epithelial cell adhesion molecule expressed exclusively in epithelial tissues. Since EpCAM is expressed only in epithelial tissues there is considerable mosaic expression of MSH2 hypermethylation in EpCAM deletion carriers (52). This can lead to complications in evaluating IHC results.

Mutations identified in MMR genes are classified as pathogenic (deleterious), benign or as a variant of uncertain significance (VUS). These variants are usually single nucleotide substitutions causing a missense mutation or a single nucleotide variant located near a splice consensus sequence. Factors such as the frequency of the variant in the normal population, family segregation studies, the nature of the missense substitution (for example, a nonconservative substitution involving an evolutionarily conserved amino acid), and in silico tools (such as SIFT or Polyphen) may be helpful in classifying a VUS. RNA analysis can be useful in determining the significance of splice variants as well as in silico software to predict the effects of variants on RNA splicing (SpliceSite Finder) (53).

Epigenetics and Lynch syndrome (LS)

Epigenetics refers to heritable changes in gene expression that occur independently of changes in the DNA sequence (54). Epigenetic mechanisms often involve DNA methylation and chromatin remodeling through histone modifications and non-coding RNAs (such as microRNAs). A constitutional epimutation is an epigenetic aberration, found in all cells that usually involve promoter hypermethylation that leads to silencing of the gene. The identification of epimutations in MLH1 and MSH2 in LS families has brought to light the important role of epigenetic mechanisms in cancer development. Epimutations may be primary or secondary. Primary epimutations have been identified in MLH1 and they show

unpredictable, non-Mendelian inheritance patterns varying from apparent heritability to reversion to the normal state in successive generations. Secondary epimutations result from indirect genetic alterations that activate epigenetic factors to cause gene methylation and silencing. A classic example of a secondary epimutation is the EpCAM deletion, which results in a read-through transcript that induces hypermethylation of the MSH2 gene. Secondary epimutations have also been identified in the MLH1 gene. In contrast to primary epimutations, secondary epimutations in MLH1 and MSH2 show Mendelian autosomal dominant inheritance because they result from genetic alterations. Future challenges involve understanding the basic mechanisms involved in primary (or reversible) and secondary (or dominant) epimutations. Until the mechanisms are more clearly defined, family members of individuals with epimutations should be offered methylation testing to determine their carrier status (55,56).

There currently are no methods of detecting carriers of LS, short of searching for specific mutations once an affected family member has been tested. Universal LS screening propels genetics into the primary care arena, by identifying individuals with a hereditary predisposition towards LS. NCCN 2014 endorses Universal Lynch screening either by testing all tumors or all <70 plus those who meet the Bethesda criteria since guidelines such as the Amsterdam and Bethesda criteria fail to identify 50% of individuals with LS (24). The cost effectiveness of Universal LS Screening is further realized with the expansion of cancer risks for family members (57).

Familial adenomatous polyposis (FAP)

FAP is an autosomal dominant disease caused by mutations in the APC gene located on chromosome 5p22.2 and characterized by large numbers of adenomatous polyps (hundreds to thousands) throughout the colon. A variant of FAP called attenuated FAP (AFAP) is characterized by less than 100 colon polyps and the onset of polyposis and cancer occurs later than in FAP. The APC gene encodes a large protein (2,843 amino acids) with multiple cellular functions including its role in the wnt-signaling pathway, a role in intercellular adhesion and in microtubule assembly and stabilization. A number of variable features may be associated with FAP including congenital hypertrophy of the retinal pigment epithelium (CHRPE, 60% of families), upper gastrointestinal tumors (especially periampullary carcinoma), epidermoid cysts, osteomas and desmoids

tumors (58,59).

Over 1,500 mutations leading to FAP have been identified in the APC gene, the majority being nonsense (28%) or frameshift (small deletions, 46% or insertions, 10%) mutations producing a truncated and defective protein. Gross deletions or duplications of the APC gene account for about 10-15% of mutations. In addition, the new mutation rate for APC is reported to be about 20%. In about 30% of FAP cases mutations involve codon 1061 and codon 1309; germline mutations rarely occur beyond codon 1600. However, mutations associated with AFAP often occur in the 5'-part of the gene (exons 1-4) and in the 3' part of exon 15 (49). Standard APC gene testing involves full sequence analysis; if no mutation is identified testing for gross gene deletions or duplications is done by MLPA analysis. A mutation is detected in about 80% of classic FAP patients by sequencing and in an additional 10-15% of patients a mutation is detected by MLPA analysis. The penetrance of APC mutations is almost 100% (49).

The significance of missense variants (VUS) in the pathogenesis of FAP is unclear. One particular missense variant found in about 6% of those of Ashkenazi Jewish ancestry is associated with a several-fold higher risk for development of colon adenomas and CRC. Testing for this variant is appropriate only for people of Ashkenazi Jewish ancestry and early screening is recommended for those who test positive (49,59).

MUTYH-associated polyposis (MAP)

Some patients who present with a low number of polyps without affected parents may have the syndrome of MAP. MAP shows autosomal recessive inheritance and results from biallelic mutation of the MutY, E. Coli, Homolog of (also referred to as MutY, E. Coli, Homolog of) gene which functions to remove adenine residues mispaired with 8-hydroxyguanine in DNA (49). The majority of the mutations detected (over 100 to date) are point mutations (nonsense, missense, or small insertions or deletions). Two common missense mutations (p.Y165C and p.G382D) account for about 70% of the mutant alleles in a Northern European population. About 1-2% of the general population is thought to carry a MUTYH mutation (49).

Chemoprevention

In addition to endoscopic surveillance, chemoprevention of CRC appears promising. Non-steroidal anti-inflammatory agents were shown to reduce the occurrence of adenomas in FAP (60,61). Recent reports of ongoing studies show the promise of Cox-1 inhibitors (Asprin) in CRC prevention (62,63). The Pharmacogenomics of aspirin metabolism shows promising results (64).

Summary

The successful incorporation of genetics in CRC prevention and treatment has the potential to greatly reduce the burden of disease. Ideally, healthcare providers must include detailed extended family histories, and discuss all the technical information currently available. A team approach involving clear communication between the healthcare specialists is optimal. Technological advances help to improve personalized care through triage and stratification but risk alienating patients' understanding due to the increased use of scientific jargon. The goals of genetic counseling are to educate the individual and their family regarding the natural history of the disease and hereditary predisposition, reduce anxiety related to that risk, and provide the tools aimed at prevention. It is our hope that this two-part manuscript will enable more providers to become a partner in CRC prevention.

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