Undefined familial colorectal cancer

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Abstract

Colorectal cancer (CRC), one of the most common cancers of the world, is actually a spectrum of several subtypes, with different molecular profiles, clinico-pathological characteristics and possibly separate pathways of progression. It is estimated that in approximately 25%-35% of cases, a familial component exists, so they are classified as familial CRC (fCRC). However the known hereditary CRC syndromes justify only up to 5%. The rest are attributed to some inherited genetic predisposition passed to offspring through low-penetrance genes, which in the proper environmental setting can bring on tumorigenesis. Furthermore, part of the familial clustering may be attributed to chance. Because of the complexity regarding the etiology of CRC, the clinician is sometimes faced with obscure patient data, and cannot be sure if they are dealing with fCRC or sporadic CRC. The elucidation of what is going on with the as yet "undefined" portion of CRC will aid not only in the diagnosis, classification and treatment of CRC, but more importantly in the proper adjustment of the screening guidelines and in genetic counselling of patients.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common cause of cancer death in the world. This is attributable to both its relationship to many environmental factors common in everyday life (especially in developed countries) and to its complex genetic background, which seems much more complicated than was once believed and is just starting to unravel.

Traditionally, CRC has been broadly classified into sporadic and inherited, the first being the result of successive spontaneous mutations and the latter being the ultimate outcome of a germline mutation that initiated the carcinogenic process. Familial adenomatous polyposis (FAP), Lynch syndrome (originally described as hereditary non-polyposis colorectal cancer or HNPCC) and several other syndromes fell in the latter category. However a significant percentage (20%-25%) of patients with CRC have some features of hereditary cancer but cannot be classified into any of the currently recognized syndromes since they don't feature the classic "single-gene defect" genotype. Considered retrospectively, it is possible that these patients have had some increased risk attributable to inherited mutations which were not sufficient to trigger

carcinogenesis on their own, and are thus put in the category of familial CRC (fCRC).

In this article, we briefly review the latest literature concerning the classification of CRC and try to explain the basis of familial CRC. Further progress on this would be valuable for genetic counseling and preventive decision making; on one hand, patients at increased risk could be advised to take the appropriate prophylactic measures, while on the other hand overdiagnosis of hereditary CRC syndromes and subsequent unnecessary actions could be avoided.

DEFINED HEREDITARY SYNDROMES AND PATHWAYS

In 1990, Fearon *et al*[1] proposed a very attractive model concerning the putative molecular events underlying the progression of colorectal cancer through several morphological stages known as the "adenoma-carcinoma sequence". Subsequent research refined this and generated the "traditional pathway" model. Approximately 80%-85% of CRCs develop through this pathway, which has also been called the chromosomal instability (CIN) or "suppressor" pathway[2].

CIN is the one of the two categories of genomic instability, and is a state in which a pre-cancerous clone has developed a cellular environment permissive of future mutation; this gives the advantage of accumulation of strategic mutations and accelerated carcinogenesis. In CIN the genetic events necessary for cancer development are favored by chromosomal abnormalities (aneuploidy). The early stages of this pathway are associated with mutations in APC (5q) and KRAS (12p) or deletions of their respective chromosome parts, while in later stages loss of heterozygosity (LOH) at the DCC/SMAD4 (18q) and P53 (17p) loci appears to be very frequent.

Adenomatous polyposis coli (APC), the gene mutated in FAP and related syndromes (Attenuated FAP, Gardner's and Turcot's syndrome), is an important regulator of growth, differentiation and apoptosis. Mutation or loss of APC may contribute to the cell's malignant potential in two ways. Firstly the Wnt cascade[3], important in maintaining the tissue-specific stem cell compartment[4], becomes more active favoring the initiation of the aberrant crypt foci (ACF). In addition to that, disturbance of β-catenin signaling, which among others participates in intercellular communication through interaction with cytoskeletal components (mainly E-cadherin), may give the cell some independence from inhibitory contact signals. Finally the APC gene is important in promoting correct chromosomal alignment and subsequent chromosomal segregation during mitosis, so that APC deficient cells can bypass the metaphase checkpoints without halting and undergoing apoptosis in case of chromosomal abnormalities[5,6].

The importance of the above is obvious from the fact that APC aberrations are found in 70%-80% of CRCs, irrespectively of whether they are familial or sporadic, while in the rest of the CRCs which retain a completely normal APC protein, mutations in other components of the Wnt pathway are found (β-Catenin, Axin, Conductin, GSK3β, TCF4 *etc*)[7]. APC is a large gene, so aberrations can occur in multiple ways, with variable effects[8]. Hence, mutations that do not completely abrogate APC's function lead to a less marked phenotype called "Attenuated FAP" (AFAP). Furthermore, mutations of MutYH - a gene encoding for a DNA glycosylase involved in base excision repair - increase the rate of G:C→T:A transversions[9]; this particularly affects the APC gene, leading to the MutYH-associated polyposis syndrome (MAP) - an autosomal recessive version of AFAP. MutYH inactivation is also associated with activating mutations of Kirsten Ras (KRAS), a proto-oncogene which encodes for a G protein that is an integral part of the Mitogen-Activated Protein Kinase (MAPK) pathway.

The remaining 15%-20% of CRCs develop through another pathway which comprises Microsatellite Instability (MSI), the second type of genomic instability. MSI is characterized by expansion or contraction of nucleotide repeat sequences which stems from dysfunction of the cell's mismatch repair (MMR) system. This can be the result of germline mutations in MMR genes (e.g. MSH2, MSH6, MLH1) as happens in Lynch Syndrome, or of epigenetic silencing through methylation of their promoters (especially of the MLH1 gene). This alternative pathway (also called the "mutator" pathway) is characterized on the one hand by frameshift mutations in critical genes with coding microsatellite sequences (such as TGFβR2, BAX, TCF4, RIZ, IGF2R) and on the other hand by a generalized tendency to point or frameshift mutations.

Through the progress made lately on epigenetics it has been established that a great fraction of CRCs are characterized by global hypomethylation of the genome and concurrent hypermethylation of cytosine bases at the promoter regions of strategic genes[10]. As a result oncogenes are switched on while tumor-suppressor genes are switched off. Hence the CpG Island Methylator Phenotype -positive [CIMP(+)] cancer was defined[11,12], calculated to occur in approximately 30% of CRCs[13]. CIMP does not perfectly overlap with MSI, but it encompasses a big part of it (Figure 1). It also partly overlaps with CIN cancers and is a feature of about half the sporadic CRCs.

The most noteworthy thing about CIMP is the fact that CIMP(+) CRCs do not usually progress through the adenomatous polyps pathway (AD) as in FAP, but instead through a novel sequence termed the serrated neoplasia pathway (SP)[14,15]. SP is mainly characterized by three alterations at the molecular level, namely activation of the MAPK pathway, inhibition of apoptosis and disturbances in DNA methylation. The first seems to occur through mutation of KRAS or BRAF (the v-raf murine sarcoma viral oncogene homolog B1) and sometimes through downregulation of the Ephrin B2 (EPHB2) gene. In fact KRAS and BRAF - two molecular switches acting
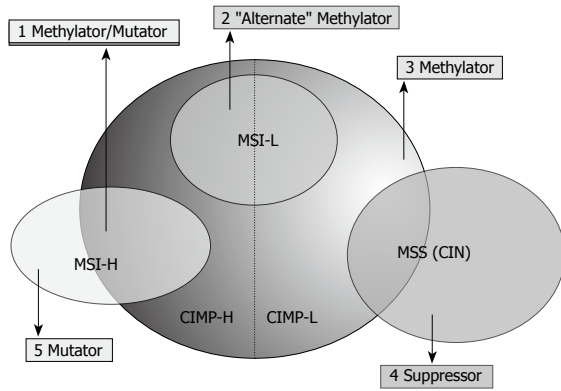
Figure 1 The spectrum of the molecular profile of colorectal cancers. Groups 1 to 5 are estimated to amount for 12%, 8%, 20%, 57% and 3%, respectively. CIN: Chromosomal instability; MSS: Microsatellite stable; MSI: Microsatellite instability; CIMP: CpG island methylator phenotype; -H: High; -L: Low.

Table 1 Amsterdam Ⅰ and Ⅱ criteria and Bethesda (revised) guidelines	
Term	**Criteria**
Amsterdam Ⅰ [65] (All criteria must be met)	At least three 1st degree relatives with CRC
	At least two successive generations affected
	At least one family member diagnosed below age 50
	FAP excluded
Amsterdam Ⅱ [65] (All criteria must be met)	As for Amsterdam Ⅰ except that CRC may be substituted by any of: CRC, cancer of the endometrium, small bowel, ureter, or renal pelvis
Bethesda (revised)[66]	Test for MSI if: (Any criterion met)
1	CRC diagnosed below age 50
2	Presence of multiple CRC or HNPCC-related cancers[1] (synchronous or metachronous)
3	CRC with MSI-H-related histology[2] diagnosed below age 60
4	CRC or HNPCC-related cancer in at least one 1st degree relative, diagnosed below age 50
5	CRC or HNPCC-related cancer in at least two 1st or 2nd degree relatives, regardless of age

[1]Includes cancer of endometrium, small bowel, pelviureter, biliary tract, stomach, ovary, pancreas, and brain (mainly glioblastoma multiforme); [2]Tumor infiltrating lymphocytes, Crohn-like reaction, mucin/signet ring cell differentiation, medullary growth pattern.

sequentially in the MAPK pathway - appear to be mutated in an almost mutually exclusive way in CRCs.

Serrated polyps are believed to be the original lesion from which CRCs can evolve *via* the SP. They are common in the elderly and include subtypes such as aberrant crypt foci, conventional hyperplastic polyps, mixed polyps, serrated adenomas and sessile serrated adenomas (SSA), all being biologically distinct as signified by differences at the molecular level[16]. SSAs however often have an aggressive behavior and may be part of the Hyperplastic Polyposis Syndrome (HPS, proposed to be renamed to serrated adenomatous polyposis syndrome), defined by Burt and Jass[17]. They may progress through dysplasia to serrated adenocarcinoma and show a predilection for the right colon in middle-aged females. At the molecular level, they display a high level of BRAF mutation instead of KRAS. On the other hand, SSAs may represent the precursor lesion to sporadic MSI carcinomas[18].

Several hereditary forms of CRC are well recognized. The classic FAP is defined by the presence of hundreds (or more) of colorectal adenomas, which will almost certainly undergo malignant transformation in the future. Attenuated FAP and MutYH-associated polyposis are both defined by the genetic defects described above. Morphologically, they are characterized by a milder phenotype than FAP and account for approximately 15% and 35%, respectively, of a heterogeneous group of cases called multiple colorectal adenomas (MCRAs), which is defined as 5-100 adenomas lifelong.

On the other hand, the forms of CRC that were evidently hereditary but featured no adenomatous polyps (or rarely very few of them) were collectively classified as HNPCC. The classification was originally based on the Amsterdam Criteria (AC) (Table 1). However, after further advances of our understanding of molecular genetics, the original definition of HNPCC proved to be wrong, as it did not correspond to a single disease with distinct etiology[19]. The term "Lynch syndrome" is preferable. It corresponds to a hereditary cancerous disease that is explained by a germline mutation in

a DNA MMR gene. The AC are traditionally used, but seem to lack both sensitivity and specificity. The Bethesda guidelines (Table 1) are more sensitive and may be used for choosing whom to test for MMR deficiency, which adds to specificity as well[19,20].

Molecular-histological correlation

The recent trend has been to classify CRC according to molecular features, which are a direct consequence of the carcinogenic pathways involved in each case. A scheme proposed by Jass *et al*[21] involves five subtypes, based primarily on (1) the underlying types of genetic instability, and (2) the presence of DNA methylation. Certain clinical (location, gender) and pathological (serration, precursor lesion, tumour infiltrating lymphocytes, dirty necrosis) features interestingly fit quite well to certain subtypes. These groups may be conceived as completing a circle, rather than representing a continuous spectrum, as Jass says. The proposed classification may assist in recognition of familial cases and formulation of more appropriate criteria for fCRC, as well as effective screening of at risk patients.

Microsatellite (MS) status is graded according the Bethesda panel, which divides CRCs into three categories: MS stable (MSS) if none of them is changed, low MSI (MSI-L) if there is alteration in one of the five and high MSI (MSI-H) if two or more are altered. MSI-L CRCs show higher rates of KRAS mutation. Moreover there is very frequent methylation of the promoter of O-6-methylguanine-DNA methyltransferase (MGMT), which constitutes the genome prone to transversions and may be the mechanism to the KRAS point mutations.

Methylation status can be assessed using several panels. CIMP-positive CRCs have been subdivided into

CIMP-high (CIMP-H) and -low (CIMP-L). CIMP-H CRCs frequently harbor BRAF mutations and have a generalized increase in de novo methylation. CIMP-L CRCs almost always have KRAS mutations and a denser but less widespread pattern of methylation involving fewer genes. CIMP-H status correlates much more strongly with a positive family history of CRC.

Group 1 [CIMP-H, MSI-H, BRAF mutation, CIN(-), methylation of MLH1] or "Methylator/Mutator" is believed to originate in serrated polyps and represents the sporadic MSI-H CRCs.

Group 2 [CIMP-H, MSS or MSI-L, BRAF mutation, CIN(-), partial methylation of MLH1] or "Alternate methylator" also originates in serrated polyps. Here, MGMT loss may also occur and a synergistic effect with the loss of expression of MLH1 is possible. This group could be regarded as a "group 1/group 4 hybrid".

Group 3 [CIMP-L, MSS or MSI-L, KRAS mutation, CIN(+), MGMT methylation] or "Methylator" may originate in villous adenomas, in serrated polyps, or in mixed polyps. Along with group 2, it represents the intermediate between the MSI-H and the MSS/CIN(+), and a big part of the fCRC may fall in these two groups.

Group 4 [CIMP(-), MSS, CIN(+)] or "Suppressor" may be sporadic, FAP-associated or MAP-associated and constitutes the biggest part of the pie (approximate 57%). It evolves through the traditional adenoma pathway and APC mutation is the hallmark of this group.

Finally, group 5 [CIMP(-), MSI-H, CIN(-)] or "Mutator" originates in adenomas and is actually the Lynch syndrome, i.e. the familial MSI-H CRC.

The interrelationship between these groups and their respective characteristics are depicted in Figure 1.

UNDEFINED FAMILIAL CANCER

General perspective

The term familial cancer is not absolute. It is defined according to several panels such as the AC, which were arbitrarily set up and are mainly based upon the family history of cancer. Therefore, the percentage of familial CRCs varies according to the definition. Although twin studies report inherited susceptibility to amount at approximately 25%-35% of CRCs, the classic genetic syndromes that fit the Mendelian inheritance [FAP, Lynch syndrome, Familial juvenile polyposis, PTEN-associated polyposis (Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome) and Peutz-Jeghers syndrome] are quietly rare and account for only 3%-5% of the total[22]. The remaining 20%-30% are currently attributable to various combinations of low-penetrance mutations, which either function as modifier genes or act in concert with environmental factors to initiate and enhance the carcinogenic sequence[23]. In fact, such mutations are highly likely to be responsible for the biggest part of familiality of CRC, since individuals with high-penetrance mutations would tend to be extinguished through negative physical selection. In support of this stands the fact that there are no recognized high-penetrance genes for cancers with documented high familiality (such as thyroid cancer) evident by twin studies. This notion has been called the "common disease - common variant" hypothesis[24].

MutYH issue

As mentioned above, mutations of the MutYH gene result in the recently recognized syndrome of MutYH-Associated Polyposis (MAP), with a phenotype similar to - or even indistinguishable from - FAP and especially AFAP[25]. The hallmarks of MAP are biallelic germline mutations of the MutYH gene and hence it is inherited as an autosomal recessive disorder. These patients develop a variable number of polyps and carry significant risk of progression to CRC, as evidenced by several studies. In fact up to one quarter of cases with the FAP/AFAP phenotype who are negative for APC mutations are found to have biallelic inactivation of MutYH. Therefore it is prudent that such suspicious cases be tested for their MutYH status and if found positive, then managed with at least an appropriate screening program, such as colonoscopy every one or two years.

A reasonable question that arose is what happens with monoallelic germline mutation carriers? This question has posed considerable debate among researchers, with contradictory results occurring from several series[26,27]. The monoallelic effect seems to be of borderline statistical significance and the inconsistencies probably stem both from bias issues and from the smallness of samples, which as a result are not enough to demonstrate the effect of low-frequency mutations. Hence there is urgency for a large scale study with sufficient power. Of note, a meta-analysis of several case-control studies by Jenkins *et al*[28] showed that monoallelic carriers of MutYH do not manifest MAP but are at a 3-fold increased risk of CRC and have a cumulative risk of 8% of developing CRC by the age of 70.

Hyperplastic polyposis syndrome

Hyperplastic Polyposis Syndrome (HPS) has been reported to cluster in families and CRC occurs in the relatives of HPS individuals very often[29,30]. It is postulated to be inherited in a co-dominant mode, so in the case of heterozygotes, it may have varying phenotype, depending on the nature of the second allele. This would partly explain the role of putative alleles, which although innocuous on their own, could be detrimental in the proper setting. It would also account for part of the burden of SP CRC in the population. Although CIMP positive, carcinoma in HPS is more likely to be non-MSI high.

CRC in the context of HPS occurs through the SP, at least in a fraction of patients[16]. An interesting yet complex relationship seems to exist between the molecular mechanisms. Although KRAS or BRAF mutations occur early in the setting of HPS, they are not protumorigenic on their own and may lead to replication arrest and even apoptosis. However, in the presence of CIMP, the situation is reversed. This probably results from the silencing of critical genes implicated

in apoptosis and cell cycle control through promoter methylation. An underlying mechanism directing the methylation of genetic loci that bear specific motifs has been suggested[31]. A vicious circle then ensues where genetic alterations accumulate and methylation patterns are disturbed, the cell becomes even more independent of growth-inhibitory and apoptotic signals and so on.

What is special then about HPS patients? Apparently they have an inherited tendency to CIMP, which creates a field cancerization effect upon their colon (and possibly other organs) through inappropriate hypermethylation[32]. CIMP acting in synergy with a somatically acquired BRAF mutation opens the door to CRC *via* the SP. The process is compounded by environmental exposure; smoking in particular has been proven to significantly increase the risk for CRC in the presence of CIMP and BRAF mutation[33].

"Familial Colorectal Cancer Type-X" syndrome

Lindor *et al*[34] introduced the term "Familial Colorectal Cancer Type-X" to encompass all the CRC incidents with evidence of familiality based on any number of pedigree and/or laboratory criteria (including the AC) that are not Lynch Syndrome (i.e. do not have any evidence of MMR deficiency). This is not an actual disease entity, but rather a group that includes all the familial non-MMR-mutated CRCs, which was devised to compensate for our lack of understanding for the exact etiology, hence the "X". In a recent article, Jass explains why HNPCC is an unfortunate term and further clarifies the application of fCRC Type-X[19]. However fCRC "type X" patients have a lower incidence of CRC than actual Lynch syndrome patients, while the risk for extra-colonic cancers may not be increased at all. Moreover, the age of onset of CRC may be significantly different, since Lindor *et al*[34] found the mean age of diagnosis to be greater by 12 years in the "type X" group. Hence a patient should not be loosely tagged as "type X" if not all the hereditary CRC syndromes have been excluded. The checklist should also include conditions like Juvenile Polyposis, Hereditary Polyposis Syndrome *etc*, which, although rare, are attributable to specific mutations and have specific pathologic characteristics[35].

A big part of the multiple colorectal adenomas (MCRA) group mentioned before is not explained by the as yet defined syndromes (AFAP/MAP), but family history of CRC is present. Therefore it may fall under the fCRC Type-X heading. Currently, this is attributed to germline variation, which can accelerate carcinogenesis under the appropriate circumstances. A very recent study on German patients of this category (i.e. family history in the absence of a recognized CRC syndrome) found increased prevalence of MutYH mutations, compared to sporadic CRC or controls[36]. This finding, together with the results of the meta-analysis mentioned above[28], imply that inactivation/loss of one MutYH allele may be responsible for part of the MCRA hereditary predisposition to CRC. Variations in genes of the Wnt pathway may also account for part of the MCRA, since β-catenin is significantly overexpressed in these patients[37].

Of course we must not overlook the possibility of part of the familiality of CRC in the "X" syndrome being attributable to common environmental exposure. Several studies have shown that in various settings there are strong associations between shared lifestyle factors (e.g. smoking and alcohol intake) and family history of CRC[38]. Hence, in some cases, family history may actually be a confounding factor, while the real culprit for increased CRC risk is the shared environment. However these studies have many inconsistencies as well as many limitations regarding the patients' characteristics (gender, age, *etc*), warranting further evaluation of their results.

Upon recognition of a suspicious pedigree, the key clinical question to be answered is whether we are dealing with a hereditary cancer or several random sporadic events. This will guide the management of both the patient and his asymptomatic relatives. Answering this question is not feasible in many cases, because the causative mutation is not known and hence the desirable genotypic investigation cannot be done. However, the common disease - common variant hypothesis implies that if we find a way to screen for the low-penetrance mutations, then maybe we will be able to predict - at least partly - the risk of individuals of developing CRC. Among others, this could potentially contribute to increasing the compliance of patients to lifestyle modifications as preventive measures.

Since low-penetrance mutations usually take the form of single nucleotide polymorphisms (SNPs), the desired analysis may be accomplished by conducting genome scans for SNPs. A prerequisite for this is to recognize SNPs that confer increased risk for CRC, and at a later stage to quantify the risk and find the best-benefit treatment.

Role of polymorphisms

Lately much research is being conducted on discovering novel low-penetrance mutations, with quite a few definitive and in some cases with confusing results. One reason for that is the use of association studies (a type of case-control study), which do not have sufficient power. As a result, some eventually prove to be inaccurate, since they cannot be reproduced in subsequent studies. For example the much-mentioned TGFβR1 × 6A variant of the TGF-β type 1 receptor has been found in several studies to be responsible for a slightly increased risk for CRC (20%) which is also dose-dependant (homozygotes > heterozygotes) and is important because it is quite common in the general Caucasian population, raising the attributable risk to 1.2%[39,40]. However a very recent study on 1042 CRC cases *vs* 856 controls found the relative risk to be only 1.05, which was not significant and concluded that there is no association of the polymorphism with increased CRC risk[41].

An interesting point is that several suggested polymorphisms result in small repeats of a nucleotide. Such oligonucleotides are prone to replication errors through DNA polymerase slippage and constitute a form of genomic instability. The most thoroughly studied polymorphism is probably the I1307K of the APC

gene, found in a significant percentage of the Ashkenazi Jews[42]. This change creates a stretch of eight adenines (A8) instead of the normal A3TA4, which is vulnerable to insertions or deletions that create a truncated APC protein. A second polymorphism in the APC, the E1317Q, may increase the risk for MAP, although still unproven. Given the crucial role of the Wnt pathway in colorectal carcinogenesis - which is virtually always deregulated in CRCs - we should expect polymorphisms affecting genes encoding for other molecules of the cascade also to modify the risk[8].

Many other gene polymorphisms have been suggested as candidate low-penetrance mutations. They correspond to genes participating in many different functions, including metabolism, cell cycle control, maintenance of DNA integrity and immune response. Polymorphisms in glutathione-S transferase theta (GSST1) and N-acetyl transferase (NAT2) genes impact on the biotransformation of carcinogenic substances, possibly resulting in decreased detoxification and increased presentation of carcinogens to crypt cells[43,44]. The enzyme 5,10-methyletetrahydrofolate reductase plays a pivotal role in the metabolism of nucleotides and thus in the repair of DNA errors. Homozygotes for valine at position 667 (MTHFR × 667V) have a protective advantage, because the decreased levels of methylene-THF interfere with thymidylate biosynthesis, leading to deoxyuridylate pool imbalances[45]. A similar effect was found recently with homozygosity for valine at position 222 (MTHFR × 222V)[46]. However, a newer study in the Japanese population demonstrated that haplotypes that lead to reduced MTHFR activity are associated with promoter hypermethylation and subsequent increased risk of CIMP(+) CRC[47]. The latter finding is supported by the observation that systemic DNA hypomethylation may occur in the case of inadequate dietary folate supplementation, which is at least partially reversible when folate intake is restored to physiologic levels[48,49]. The whole case here represents a nice example of the intricate interaction between genetic polymorphisms and environmental exposure, as well as how strikingly different the inter-population variations can be.

Genes involved in DNA repair comprise another category believed to modify the risk for CRC through subtle changes in their sequences[50]. Many polymorphisms in several genes have been proposed as candidates[51] but few are proven to affect the risk in the general population. Only in the case of OGG1 and XRCC1, involved in base excision repair, and XPD for nucleotide excision repair, consistent evidence exists for association with certain types of cancer. Furthermore, the study of Webb and Rudd mentioned above raises the possibility of polymorphisms in the genes ATM and CHEK2 predisposing to CRC, through alterations in the axis ATM-CHEK2 that regulates cell cycle checkpoints based on signals for DNA integrity[46].

The gene BLM encodes a homologue of recQ helicase, biallelic inactivation of which causes genomic instability and ultimately leads to Bloom syndrome.

Heterozygotes for a particular frameshift mutation, the BLMAsh (2281 delATC TGA insTAG ATT C) in exon 10, are at approximately 2.5-fold increased risk for CRC[52]. Although heterozygotes do not appear to have increased familial clustering attributable solely to BLMAsh, it is possible that this mutation modifies the risk conferred by other variants, such as the APC × I1307K (both found in Ashkenazi Jews), and hence indirectly affects the predisposition to fCRC[53].

Another recent study confirmed the previously reported increase in risk arising from polymorphisms in the Cyclin D1 gene (CCND1) - a cell cycle control protein - and E-cadherin (CDH1), an intercellular adhesion molecule[54]. In particular, the A→G mutation in position 870 of CCND1 and the C→A variant at codon -160 of CDH1's promoter were found at increased frequency in fCRC cases, compared to sporadic cases[55]. On the contrary, this study failed to demonstrate any association between fCRC and variants of the TP53, vitamin D receptor (VDR) and ileal bile acid transporter (SLC10A2) genes. However that may not be the story with CCND1, since a study in Singapore showed that the effect of the A870G polymorphism may not act on its own, because it is modified by polymorphisms in glutathione S-transferases as well as isothiocynate intake[56]. This is another proof of how cancer risk is based on a combination of genetic predisposing factors which interact with environmental stimuli to bring on carcinogenesis.

Harvey Ras (HRAS1) is a proto-oncogene with an accompanying variable number tandem repeat (VNTR) minisatellite downstream. Polymorphisms in the latter (HRAS1 × VNTR) were shown to interact with molecules of the NF-κB family as well as other transcription regulators and hence modulate the expression of nearby genes. Some rare alleles appear to increase the risk for CRC, although this may also result from linkage disequilibrium[57].

Recent evidence also correlates SMAD7 polymorphisms with increased CRC risk[58]. SMAD7 acts as an intracellular antagonist of TGF-β signaling, deregulation of which has been shown to influence CRC progression[59,60]. Moreover, these polymorphisms are postulated to interact with other common alleles to substantially increase an individual's risk. SMAD7 status is being investigated as a tumor marker for CRC, since its amplification is associated with poorer prognosis.

Finally, polymorphisms in inflammatory response-related genes may modify the CRC susceptibility. Inflammation seems to favor tumorigenesis through various mechanisms, such as sustained DNA damage, stimulation of cell proliferation and provocation of angiogenesis. Considering that the colonic mucosa is naturally in a state of continuous inflammation because of the normal flora, factors that would aggravate this state could tilt the balance towards carcinogenesis. In a study conducted on a sample from the Greek population by our team, a significant association was found between polymorphisms of some inflammatory response-related genes and increased CRC risk[61]. Specifically, the R241G

and K469E allelic variants of ICAM-1, as well as the -174G of IL-6 increase the risk of CRC, probably *via* the aforementioned mechanism. The GG genotype at -174 of IL-6 further increases the risk. The CC genotype encoding for proline at position 12 (Pro12) of the PPARγ gene was also correlated with increased CRC susceptibility. On the other hand the Ala12 variant of PPARγ was found by others to have a protective effect. The effects of these polymorphisms may be related to the fact that PPARγ can alter the expression of COX-2. In addition to that, PPARγ can regulate the transcription of the tumor suppressor gene PTEN and hence modify several signaling pathways.

CONCLUSION

Colorectal cancer is very common nowadays. The once thought single disease is actually a spectrum of several subtypes, with different molecular profiles, clinico-pathological characteristics and possibly separate pathways of progression. New data concerning the molecular pathways of colorectal cancer evolution and the histopathology of the precancerous lesions are coming to light every day. These are expected to have decisive implications not only in the diagnosis, classification and treatment of CRC, but more importantly in the adjustment of screening guidelines in order to catch the disease early, in a perhaps curable stage.

Family history remains fundamental in the diagnosis, management and prognosis of CRC, but also in counseling and preventive interventions. However it should not be the sole guidance to diagnosis of hereditary syndromes, but should always be considered in a broader context together with clinical, pathological, molecular and biological characteristics of the tumor. The possibility of familial aggregation due to shared environment and even chance should always be kept in mind. Because of that, the family history of patients should be thoroughly obtained, but no family should be tagged as having a hereditary cancer syndrome if no solid evidence (supported by pathology and molecular features) exists[62].

The emerging concept of SNPs modifying the risk of CRC seems fascinating. The inconclusive or controversial data regarding their role may suggest that the modulation of cancer risk depends on a joint effect of multiple polymorphisms within different genes or pathways, interacting with environmental factors. In order to confirm the putative role of SNPs as low-penetrance mutations, large-scale, genome-wide linkage studies have to be conducted, using highly efficient analytical platforms and proper stratification. These will overcome most of the bias, will be much less likely to miss important variants and will thus have much more power. One such multi-center trial is currently in progress in the UK and aims to gather and analyze 20000 CRC cases[63].

MutYH-associated polyposis is just beginning to be elucidated. Screening for MutYH mutations in cases of polyposis phenotype with negative APC mutations may have special implications in the management and follow-up, as well as on the genetic counseling of relatives, especially of siblings[64].

Finally, the need for a review of the guidelines on screening and management of patients with positive family history of CRC will not be long to come. Having ruled out all the known hereditary CRC syndromes, while being confident about the familial component, the clinician has possibly discovered another "type X" patient. Based on the less severe phenotype of this entity, these patients must be treated with less aggressive measures than actual Lynch syndrome patients, such as screening every five years but no prophylactic surgery.

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