Chronic Inflammation, Colorectal Cancer and Gene Polymorphisms

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Key Words

Chronic inflammation · Colorectal cancer ·
Gene polymorphisms

Abstract

Chronic inflammation is commonly present in gastrointestinal mucosal sites at increased risk for cancer, such as in inflammatory bowel disease (IBD) or chronic gastritis caused by *Helicobacter pylori* infection. Why some patients have more mucosal inflammation than others, and why certain individuals with chronic inflammation develop cancer, are problems that have not been solved. Unlike the case for the syndromic forms of familial colorectal cancer (CRC), the risks for IBD and other forms of chronic inflammation have not been linked to highly penetrant single gene mutations. Single nucleotide polymorphisms (SNP) are variations in DNA sequence that can be linked to any phenotype (cancer, chronic inflammation, etc.) in genome-wide association studies (GWAS). CRC has been linked to several highly penetrant single gene loci, as well as multiple SNP. The propensity to develop IBD has not been linked to single gene mutations in most instances, but has been linked to SNP in the *NOD2* locus (which appear to create hypomorphic alleles for this bacterial response gene), the *IL23R* locus, the autophagy gene *ATG16L1* and a wide range of other loci including the Toll-like receptors, JAK2 and STAT3, and perhaps 70 more.

At present, the problem in predicting risk for chronic inflammation is that there are many genetic polymorphisms with relatively modest individual effects. Our challenge is to understand how the SNPs that are linked to variations in the inflammatory response interact with one another (i.e. to understand the 'epistasis' involved), and to integrate this with the variety of individual environmental exposures. This represents an opportunity for informatics science to help personalize our approach to chronic inflammatory diseases of the gut and identify those at greatest risk for cancer.

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Introduction: Nature and Nurture

Most diseases represent a failure of adaptation by the host to environmental exposures. The ability of the host to adapt is determined largely by genetic factors. There are multiple different ways that genetic variation affects disease states. Genetic variation will determine whether or not the host will develop the disease in response to environmental stress (i.e. 'penetrance'), and the differential responses to the exposure (i.e. 'expressivity'). In some instances, the nature and intensity of the environmental exposure is the principal determinant of disease, such as occurs after *Shigella* exposure in which most individuals become ill. In other instances, genetic factors are more

important in determining the response to ordinary stresses, such as with inflammatory bowel disease (IBD). This paper discusses the genetic basis of chronic inflammation, principally in the context of IBD and colorectal cancer (CRC), the latter because of the insights that carcinogenesis brings to the role of genetics in determining disease.

Traditional Approaches to the Genetic Involvement in Disease

Prior to the explosion of information on molecular genetics, simplistic approaches to disease assumed that one mutation in one gene would cause one disease. This was based upon our understanding of sickle cell disease, in which a solitary altered amino acid sequence of the globin protein was the first genetic disease that was appreciated at a molecular level. Once it became possible to explore genetics in more detail, it became clear that more than one mutation in a gene could cause the same disease, as was recognized for sickle cell disease, and later in cystic fibrosis and familial adenomatous polyposis (FAP), for example. Moreover, different mutations in single genes were found to modify the expressivity of the disease. It was further found that mutations in more than one gene can cause the same disease (e.g. mutations in the *APC* gene cause the autosomal dominant form of FAP, whereas biallelic mutations in the *MYH* gene cause a recessive form of polyposis [1]). In other instances, multiple genes may be involved in a single complex biological process and mutations in any one of these can cause a single genetic disease, as occurs in Lynch syndrome, which is caused by germline mutations in any of 4 different DNA mismatch repair genes (*MSH2*, *MLH1*, *MSH6* or *PMS2*) [2]. Moreover, the presence of gene-gene interactions and modifier genes were recognized as additional important causes of variability in disease expression. More recently, sequence variations in noncoding regions of the genome have been found to play an additional role in modifying the expression of disease. This has led to a number of genome-wide association studies (GWAS) that can identify whether any particular DNA neighborhood is associated with an altered risk for a disease.

Chronic inflammation is not characterized by single dominant, highly penetrant genes that determine the presence or intensity of the immune response. However, considerable effort has been expended looking for genes that might account for the familial clustering of diseases of immunity, as occurs in IBD and other diseases. A small

number of loci have been identified that are associated with a proportion of IBD cases, but these same genetic polymorphisms are frequently present in the absence of any identifiable phenotype.

High Penetrance CRC Genes

About a third of CRC cases occur in the context of a positive family history. Only about 3–4% of CRC can be traced to the involvement of a single, dominant gene. For example, most FAP is caused by germline mutations in the *APC* gene, which encodes a protein that is essential in regulating epithelial cell growth in the gut. The mutations in *APC* that cause FAP are quite stereotypic: all either create premature stop codons or are complete deletions of the gene. There are no missense mutations or synonymous (noncode-changing) single nucleotide polymorphisms (SNP) that sufficiently alter the function of the gene product to cause FAP. The number of polyps in the colon, the age of onset and some of the extraintestinal manifestations of FAP are determined by the location of the stop codon in the mutant gene.

A different situation occurs in Lynch syndrome, which is caused by mutations in *MSH2*, *MLH1*, *MSH6* or *PMS2*, which work together in the DNA mismatch repair complex. These genes cause familial CRC when inactivating mutations occur to any of these genes. Losses of *MSH2* or *MLH1* lead to complete loss of DNA MMR activity, and the fullest penetrance of the cancers. Mutations in *MSH6* or *PMS2* create an attenuated form of the disease because other mismatch repair proteins can replace these mutant genes [2]. The ‘expressivity’ of the disease principally depends upon which of the 4 genes is mutated, rather than the nature of the mutation or its location in the gene. Interestingly, SNP in a number of other genes have been found that modify the age of onset of CRC in Lynch syndrome. It is notable that these sequence variations do not cause disease by themselves, but rather interact with the dominant cancer-causing DNA mismatch repair gene mutation and function as ‘modifier’ genes. These include simple SNP in *Cyclin D1* (codon 242 A/G) [3], *p53* (codon 72 G/C) [4], *CYP17* (–34 T/C in the promoter) [5], *DNMT3b* (–149 C/T in the promoter) [6], *IFG-1* (polymorphism in the length of the CA repeat in the promoter) [7], *NAT2* (multiple alleles, some of which confer variable degrees of risk) [8], the glutathione S-transferase gene (null alleles modify the onset of cancer) [9] and the hemochromatosis gene [10]. In each instance, one of the alleles will decrease the median age of onset of cancer, com-

pared with the other allele, and there are often additive effects for those who have 2 copies of the high-risk allele [11]. Interactivity among gene products that modify phenotype is called ‘epistasis’.

Low Penetrance CRC Genes and Modifier Genes

Most CRCs do not occur because of mutations in genes that cause the highly penetrant syndromic forms of the disease; however, positive family histories and familial clusters of CRC are common. This could be caused by an altered gene in the family or common environmental exposures (e.g. diet) or other lifestyle issue (e.g. smoking). Several large GWAS have been performed to locate the genes or loci involved in conferring the increased risk. Numerous loci which are associated with an increased risk of developing CRC have been identified, as illustrated in table 1.

The GWAS cited here have been repeatedly confirmed by other studies. One of these loci, at 8q24.21 (SNP No. rs6983267) is of particular interest as it has been validated in studies typically involving about 1,000 subjects and >500,000 SNP. It has a reproducible odds ratio (OR) for CRC ranging from approximately 1.27 for heterozygotes to approximately 1.47 for homozygotes (i.e. the risk for CRC is increased from 27–47%, depending upon whether individuals have 1 or 2 high-risk alleles), and this SNP is not located in a gene, promoter, miRNA or pseudogene; it is located in a ‘gene desert’. These data raise speculation that this minor sequence variation may have a long-range interaction with the *MYC* gene, which is located about 335,000 bp away, which raises a new concept of how genes might interact with one another [13]. Other risk-linked loci are more complex, such as one at 9p24, which confers an increased risk for CRC of 1.46 in 1 study of familial clusters of CRC; however, it is associated with an increased risk of 1.19 in a population-based cohort from Canada and an OR of 1.17 in a combined analysis [17].

The implications of these studies are considerable. First, we appear to have identified most, if not all, of the highly penetrant, single gene contributor to hereditary CRC. There may be additional rare genetic causes of CRC and there are likely to be some unidentified recessive forms of increased risk for CRC. Secondly, several modifier SNP have been validated by their discovery in multiple GWAS. The large size of these studies, and the confirmation in meta-analyses provide a very high level of confidence that these effects are genuine (with extremely low p values for the likelihood that these associations oc-

Table 1. Genes that modify CRC risk found in GWAS

8q24.21 (rs6983267); OR for CRC = 1.27 (heterozygotes), and 1.47 (homozygotes); OR for adenomas = 1.21; also linked to prostate cancer risk; there is no gene at this locus [12]; <i>MYC</i> is about 350 kb away [13]
18q21.1 (3 SNP close to <i>SMAD7</i>); OR = 1.2 ($p < 10^{-27}$) [14]
15q13.3 (close to 3 TSGs, and at the HMPs locus); OR for CRC = 1.35 [15]
8q23.3 (rs16892766); OR for CRC = 1.27–1.43 (1 or 2 alleles) [16]
10p14; OR = 0.8 for homozygotes (protective allele) [16]
11q23 (rs3802842); OR for CRC = 1.1 ($p < 10^{-9}$) [14]
9p24; OR for CRC (AA vs CC allele) = 1.46 in CFR study [17]; 1.13 in Canadian study [18]
8q24; OR for CRC = 1.38 in CFR study [17], 1.17 in combined analysis [18]

The loci linked to increased risks of CRC, and genes in the general neighborhood are listed. CFR = Cancer Family Registry study (of familial clusters of CRC); TSGs = tumor suppressor genes; HMPs = hereditary mixed polyposis syndrome.

cur by chance), and yet for many of these, the incremental risk for CRC is in the range of 10–30% [19]. A major question that remains to be answered is how some of these SNP operate mechanistically since some are not linked to genes in which the functional importance is obvious and some are located nowhere near a gene, sometimes in ‘gene deserts’, including the 8q24 SNP [12, 14, 18].

Genetic Polymorphisms and Chronic Inflammation

There is no single gene that confers an extremely high risk for chronic mucosal inflammation such as which occurs in FAP or Lynch syndrome for CRC. However, a very large number of SNP have been identified that are linked to IBD in humans. Most carriers of the high-risk alleles do not develop IBD [20]. The genetic factors are more important for Crohn’s disease than ulcerative colitis, but both are clearly linked to a complex mix of genetic and environmental causes. To make matters somewhat more complicated, multiple single genetic models have been developed in mice that replicate IBD and none seem to have a similar degree of importance for human IBD.

Table 2. Genes and SNP linked to IBD (partial list)

<i>NOD2</i> (innate immune response to enteric bacteria): Crohn's disease [21, 22]
<i>TLR4</i> (pattern recognition molecule in the innate immune response): Crohn's disease [25]
<i>IL23</i> (cytokine receptor): Crohn's disease [27]
<i>ATG16L1/IRGM</i> : autophagy related gene: Crohn's disease [26]
5p13.1 (a gene desert, but modifies expression of the prostaglandin receptor, <i>PTGER4</i>) [29]
16p11 (near <i>IL27</i>), 22q12, 10q22, 2q27, 19q13: early onset IBD [28]
<i>FCGR2A</i> , <i>ORMDL3</i> : ulcerative colitis [31]
<i>ECM1</i> : ulcerative colitis [32]
<i>IL23R</i> , <i>IL12B</i> , HLA, <i>NKX2-3</i> , <i>MST1</i> : ulcerative colitis and Crohn's disease [31]
12q15: OR for ulcerative colitis = 1.35 [33]
1p36: OR for ulcerative colitis = 0.73 [33]
<i>IL2/IL21</i> (4q27): ulcerative colitis [34]

Genetic Involvement in IBD

The first and perhaps strongest genetic link to chronic inflammation and IBD was found in the *CARD15/NOD2* gene [21, 22]. *NOD2* is located on chromosome 16q12 and encodes a gene that responds to bacterial wall peptidoglycans and activates the MAP kinase – NF- κ B signaling cascade. Three hypomorphic alleles have been described (Arg702Trp, Gly908Arg and Leu1007fsinsC) that are linked to ileal or ileocolonic Crohn's disease, but not to isolated colonic disease. These mutant alleles are more prominently found in European than in non-European populations, and not all who carry these mutant alleles develop IBD [23]. There is a particular association between certain *NOD2* mutations and the fibrostenosing variant of Crohn's disease [24]. Curiously, excessive chronic inflammation is associated with hypoactive alleles of *NOD2*, and the mechanism of how this relates to IBD is still not entirely clear.

Associations have also been found between SNP in the Toll-like receptor 4 (*TLR4*) and IBD, which is another pattern-recognition molecule involved in innate immunity to enteric bacteria, just like the *NOD* family. TLRs encode a family of at least 10 genes, and another 13 genes interact with the TLRs to signal the inflammatory response to gut microbes. The *TLR4* 299Gly allele confers an OR of 1.3

for IBD [25], and pooled analyses from multiple studies confirm the risk to be >1.2. Still, most carriers of this polymorphism have no identifiable disease phenotype. A number of other genes have been found that confer increased risk for IBD including the autophagy gene *ATG16L1/IRGM1* [26], which adds more possibilities to the mechanisms by which chronic inflammation can be modified. A SNP linked to a subunit of the interleukin 23 receptor (*IL23R*) has also been identified as a Crohn's disease gene by GWAS [27]. Other loci on 16p11 (near the *IL27* gene), on 22q12, 10q22, 2q27 and 19q13 have been linked with early-onset IBD [28]. At least 1 SNP located in a gene desert on 5p13 has been linked with Crohn's disease [29]. At least 30 more loci have weaker associations with Crohn's disease (partially listed in table 2) [30], and there is no reason to suspect that the list will not continue to grow.

Summary: Genes and SNP Linked to IBD

It is clear that IBD has a strong genetic component, but there are multiple risk alleles involved. The murine models are only partially helpful here since there are multiple models of both Crohn's disease and ulcerative colitis: some have correlates with human disease, but others do not have any apparent links to human disease. This suggests that, as is the case with CRC, there are multiple forms of IBD and it may be necessary to consider them independently and develop interventions for each variety of the disease. Furthermore, there are almost certainly interactions among these disease loci (epistasis), which will further complicate the matter.

Resolving Complex Gene-Environmental Interactions

An interesting example of how to understand complex interactions between genes and the environment was demonstrated in a paper that uncovered an interesting relationship in a post-hoc analysis of a prospective study [35]. A study of the effect of supplemental wheat bran on colorectal adenomatous polyp recurrence showed no significant effects of the intervention. However, it was found that about 70% of the study participants had taken at least some aspirin during the period of intervention, and 688 patients were selected for additional analysis. The aspirin takers in this group of patients had 32% fewer recurrent adenomatous polyps at follow-up colonoscopy (an effect

that was substantially greater than the wheat bran intervention). Attention was focused on a SNP in the ornithine decarboxylase (*ODC*) gene, as this is the rate-limiting enzyme for the generation of polyamines which are required for epithelial cell proliferation. The SNP of interest is either a G or an A, and is located between 2 binding sites for the transcription factor MYC in the *ODC* promoter. Among the 688 individuals genotyped, 56% were homozygous for the G allele (GG), 38% were heterozygous (GA) and 6% were homozygous for A (AA). When the *APC* gene is expressed, this inhibits the transcription of *MYC* and stimulates the transcription of *Mad1*. These 2 proteins participate in the downregulation of cellular proliferation and would be expected to limit the development of an adenoma. Those study participants who had the AA allele of the *ODC* promoter had a relative risk of developing a recurrent colorectal adenoma of 0.68, independent of aspirin use (i.e. the same reduced risk as seen in the aspirin takers). However, those who had the AA allele of the *ODC* promoter and took aspirin had a relative risk of developing a recurrent adenoma that was 0.10, i.e. there was synergy between the SNP in *ODC* and aspirin use that led to a 90% reduction in adenoma recurrence. Additionally, in vitro studies determined that the presence of A alleles resulted in enhanced suppression of *MYC* together with increased expression of *Mad1*, which suppressed *ODC* expression and consequently, the production of polyamines. The aspirin effect involved induction of the spermidine/spermine acetyltransferase (*SSAT*) gene, which increased the catabolism of polyamines. The enhanced *ODC* effect (mediated by the A allele) diminished the production of polyamines, and the aspirin effect (mediated by increasing *SSAT*) increased their destruction. The combined mechanism that both reduced

production and increased turnover of polyamines produced a synergistic effect on cellular proliferation. This complex interaction provides some insight into how epistasis may function, and involves what were initially non-obvious effects [35]. This is one example of how to resolve the complexity of epistasis.

Conclusions

Chronic inflammation and cancer are complex disease processes driven by multiple interacting genes in concert with environmental influences. There are a few dominant cancer-causing genes, but there is no dominant genetic cause for IBD. There are multiple genes involved, some of which are very important for some IBD patients, and a larger number of genes and SNP that confer a modest increase in risk. There is a very high likelihood for epistasis among these genes, an area that has only recently been explored. The development of rational intervention strategies based upon what we currently know about the genetics of chronic inflammation is limited. We need to know much more about how these genes interact with one another and with a variety of environmental stresses in order to move ahead. Because of the powerful research technologies currently available, this represents a tremendous opportunity for progress.

Disclosure Statement

This work was supported by the Baylor Foundation, the Baylor Research Institute and the Sammons Cancer Center of Baylor University Medical Center. C.R.B. is the recipient of NIH Grant R-01 CA72851.

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