Genetics of Inflammatory Bowel Disease

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Abstract— At the current time, causal factors for ulcerative colitis and Crohn’s disease are unknown. Still, there is strong evidence to suggest that genetic predisposition play a major role in the development of IBD. The purpose of this review is to highlight the important genetic aspects of IBD.

Keywords— DNA methylation, genomics, ulcerative colitis and Crohn’s disease

I. DNA METHYLATION IN INFLAMMATORY BOWEL DISEASE (IBD)

The addition of methyl moieties on CpG dinucleotides generates the highly stable and conserved covalent modification of DNA. However, these epigentic characteristics are not subjected to dynamic changes at specific time points (such as embryonic development) and during perturbed cellular homeostasis (such as enhanced cellular stress and disease onset). Therefore, these temporal modifications will greatly influence the development of inflammatory bowel disease (IBD).[1-8]

Throughout germ cell specification and post-fertilization, 5mC were subjected to de novo erasure and consecutive reprogramming.[9-11] This wholesale DNA methylation reprogramming leads to the formation of parental specific gene expression, including X-linked effects and genomic imprinting, where gene expression is mainly joined by specific parental allele. There is evidence of parent-of-origin effects in IBD.[12-15] Akolkar et al.[15] have first determined a familial occurrence of IBD. The clinical data in this study, analyzing 135 families, indicated that offsprings of IBD affected mothers were at higher risk of developing CD than those of IBD affected fathers (p=0.00001). Parent gender did seem to play a role in IBD susceptibility and in the genetic imprinting process, at least partly, by DNA methylation. In a recent study, Fransen et al.[16] have noted limited evidence for genomic imprinting effects of IBD susceptibility genes. Their analysis comprised 28 IBD susceptibility gene locus and found that IL12B, PRDM1 (PR domain containing 1) and NOD2 (nucleotide-binding oligomerization domain containing 2; L1007fs variant) have genomic imprinting effect. Schaible et al. have indicated that offsprings of female mice fed with methyl donor supplements (folic acid, betaine and vitamin B12) had an impressive susceptibility towards dextran sulfate sodium (DSS)-induced colitis, in contrast to control mice with regular diet. Colonic mucosal DNA methylation profile changes and prolonged gene expression changes have also indicated these effects, together with differences in bacteria microflora when compared to control mice.[17] Thus, a better characterization of imprinting and parent-of-origin effects and mechanisms could be used as a clinical risk predictor of IBD for offsprings of IBD susceptible parents.[9]

DNA methylation dynamics is also activated when colonic cellular homeostasis is disturbed, for instance during oxidative stress, resulting in global loss or gain in DNA methylation. Common phenomenon in IBD and IBD-associated colorectal cancer (IBD-CRC), oxidative stress and damage are mainly determined by the reactive oxygen species (ROS) induced by inflammatory cells.[18-22] Oxidative cell damage generates recruitment of DNMT1 to the affected chromatin, resulting in a complex containing DNMT3B and members of the polycomb repressive complex 4, including SirTuin-1 (SIRT1), enhancer of zeste homolog 2 (EZH2), and embryonic ectoderm development (EED), to restore DNA methylation pattern after DNA repair.[23] These basic components move from non-GC-rich regions to GC-rich regions. This finding was confirmed by an in vivo model of colitis, where inflammation and tumorigenesis were induced by infection with human commensal enterotoxigenic Bacteroides fragilis (ETBF) in a mouse model of adenomatous polyposis coli in Multiple intestinal neoplasia (Min) mice. This model might explain how, during disease onset and within the same cell, some specific genes are hypermethylated, while other loci are hypomethylated.[9, 23]

Even if there is a well-established consensus according to which DNA methylation is a highly stable modification on DNA in steady-state, this conventional perspective was chanced. Kangaspeska et al.[24] indicated that estrogen receptor α (ERα) induces transcription waves of its target promoter that includes active and cyclical demethylation and remethylation throughout transcriptional activation. The status of DNA methylation was quantified by glutathione S-transferase tagged methyl binding domain (GST-MBD) pull-down assay, indicating a 100 minute-cycle at the ERα

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target pS2 gene promoter. Particularly, DNM3A and DNM3B deminated methylated CpG, leading to base-pair mismatch later repaired by base-excision machinery. Thus, these two reports indicated for the first time the cyclical methylation-demethylation association with transcription, a process mediated by DNMTs, proteins which were previously strongly connected only with methylation and not with demethylation. This finding was also validated in other promotors, including ERα, trefoil factor 3 (TFF3) and potassium inwardly-rectifying channel, subfamily J, member 8 (KCNJ8). What is interesting is that these selected validated genes have previously been involved in different studies of IBD and IBD-CRC in patients or animal models.[25] TFF3 is produced by intestinal goblet cells forming part of the enteric mucus layer and playing a role in epithelial repair and restitution. TFF3−/− mice are less reactive towards mounting repair response during colonic injury generated by hypoxia, chemical and radiation stress.[26] Another validated candidate is potassium inwardly-rectifying channel, subfamily J, member 8 (KCNJ8, also known as KIR6.1), which produces the pore-forming subunit of the ATP-sensitive potassium channel (KATP) hydrogen sulphide (H2S), generated by colonic smooth muscles, neurons and other enteric cell types, activates and opens KATP channels in a 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced murine colitis model. Likewise, in TNBS-induced colitis in rats, H2S production and effects are correlated with colitis resolution.[27] However, it is not well known if the cyclical demethylation-demethylation process affects the pathogenesis of IBD, this aspect requiring further thorough studies.

**GENOME-WIDE DNA METHYLOME PROFILES IN IBD**

Multiple reports on genome-wide DNA methylome analysis assessing the pathogenesis of IBD have been provided by recent advances on genomic and epigenetic technologies at the omics level.[28-32] IBD analysis provided information for the development of new diagnostic and therapeutic strategies. Genome-wide altered methylation pattern enrichment around GWAS identified loci has been identified.[33-36] Moreover, methylome profiling might also determine the differences between UC and CD in terms of etiology and pathogenesis.

Several authors have profiled the methylome of whole blood genomic DNA from 21 ileal CD patients and 19 healthy controls. A number of 1177 CpG differentially methylated regions have been identified. On this list, 35 genes corresponded to previous GWAS identified CD loci, including NOD2, TNFa and caspase recruitment domain family, member 9 (CARD9). Gene comparison indicated that differentially methylated CpG sites are located within 25-100 kb of the 71 previously identified GWAS CD loci. Gender, environment and individual lifestyle (for example, non-smoking and immunomodulatory therapy status) factors were considered for cohort selection in this study, as they have strong influence on determining IBD and epigenetic alterations. This fact reflects in the high discordance rate of CD (68%) and UC (85%) in monozygotic twins, with identical genomes.[37] The arising issue is how do these identical genomes in monozygotic twins result in different phenotypes.[9] A recent report on 20 monozygotic twins conflicting UC has assessed the genomic profile based on three-layers of genome-wide scans, including transcriptome profiling, genome-wide methylation variable positions (MVPs) and genome-wide differentially methylation regions (DMRs). This study identified 61 disease loci characterized by different gene expression profiles and at least one methylation variable position (MVP) or differentially methylated region (DMR) position within 50 kb from the transcription start site. Hypomethylation was discoverde in the promoter regions of these hits, while hypermethylation was found in gene-intronic regions.[38] Nevertheless, none of the 61 loci superposed with previously noted 47 UC GWAS risk loci.[39] However, environmental factors and lifestyle definitely influence the pathogenesis of IBD and offer direct evidence to understand how identical genomes from monozygotic twins can trigger different susceptibility to IBD. Inflammatory bowel disease normally occurs during young adulthood, with the peak of onset at around 15-30 years of age.[9, 40-42]

**II. GENETICS AND PATHWAYS IN IBD: NOD2**

Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) was the first gene associated with IBD, involving several other genes that interact epistatically with NOD2 signalling. NOD2 recognizes muramyl dipeptide (MDP), the peptidoglycan product which modulates both innate and adaptive immune responses.[43-47] MDP stimulation triggers autophagy, which controls bacterial replication and antigen presentation, and influences dendritic cells together with TLR ligands, promoting TH17-cell differentiation.[47, 48] NOD2 can also influence immune tolerance. This impact is damaged in patients with Crohn’s disease-associated NOD2 mutation 3020insC. Moreover, NOD2 can take part in different MDP-independent pathways, such as the control on T-cell response and type I IFN response to single-stranded RNA (ssRNA) stimulation, attesting the presence of gut microbial ssRNAs and their immunomodulatory properties.[49] There is great variation between cell types regarding the relative contributions of these cytosolic MDP-sensing pathways. There is need for further studies to reveal the effect of disease-associated NOD2 alleles in different cell-specific programs, and to explain the precise influence of NOD2 in IBD. NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs) are other families of innate immune receptors related to intestinal inflammation and immunity.[49] They identify microbial patterns or damage-associated molecular patterns and are able to initiate the inflammasome. Therefore, it is necessary to appropriately regulate these pathways for intestinal homeostasis. For instance, mouse knockout studies of Nlrp3 or RIG-I (also known as Ddx58) indicate higher susceptibility to experimental colitis.[47] On the other hand, continuous overactivation of NLRs might have harmful effects, as shown by activating mutations in NOD2 and NLRP3, resulting in Blau
syndrome and cryopyrinopathies.[49]

III. DNA METHYLATION IN INFLAMMATORY BOWEL DISEASE (IBD)

Wu et al.[50] were the first authors noting microRNA (miRNA) expression in colonic mucosa samples from IBD patients in 2008. Eleven miRNAs were identified, differentially expressed in active UC vs controls demonstrating an inverse relationship between macrophage inflammatory peptide-2α (previously shown to be involved in IBD and mir-192).

Several recent studies[51-55] identified modified miRNA expression in IBD tissue, without subsequent functional analyses. Takagi et al.[49] formed a group of 12 controls and 12 active UC patients and noted that miR-21 and miR-155 were significantly upregulated in inflamed colonic UC tissue. In another study, Wu et al.[51] analyzed 467 miRNAs expression in sigmoid CD patients and in patients with active terminal ileal CD. They identified five miRNAs associated with active sigmoid CD, and four miRNAs significantly higher in active ileal CD, when compared to controls. A similar study by Fasseu et al.[56] further assessed the expression of more than 300 miRNAs in colonic tissue samples of UC and CD patients using quantitative real-time polymerase chain reaction analysis. Some miRNAs were differentially expressed depending on disease type, but large in number in both groups. A series of eight miRNAs (miR-26a, miR-29a, miR-29b, miR-30c, miR-126*, miR-127-3p, miR-196a, and miR-324-3p) was defined describing quiescent IBD vs controls, and other 15 miRNAs were found to differentiate between quiescent UC and CD (n=16).[56] These three studies indicate the potential use of miRNAs as biomarkers and the possiblle development of miRNA profile-based diagnostic tools.

The semi-invasive approaches with diagnostic purposes have used whole blood instead of colonic tissue. Wu et al.[57] performed a microarray-based study on whole blood from patients with IBD and discovered a panel of differentially expressed miRNAs that allowed them to differentiate between active IBD subtypes themselves and controls. Paraskevi et al.[58] have identified that 11 circulating miRNAs differentially expressed in blood samples from active CD than in controls, and described six miRNAs that were significantly higher in active UC than in healthy controls.

Despite being preliminary, these studies demonstrate the possible development of a necessary semi-invasive test based on differentially expressed peripheral blood miRNAs.

IV. GENETIC PREDICTORS OF IBD COURSE

Previous to genome-wide association studies, the role of genetic factors in IBD severity had been assessed by comparing familial and sporadic IBD.[59] Even if related to IBD, the severity of CD was not affected by family history.[41, 60] The risk of subsequent CD after ileal pouch-anal anastomosis (IPAA) is higher in case of a family history of CD.[61] The labeling of genetic prognostic factors in IBD is attractive because they are already present at the onset of the disease, even before it. Their long-term stability is highly beneficial, as this feature is not common in other possible predictive factors, such as clinical parameters or serologic markers.[61] Nevertheless, even if more and more susceptibility loci have been identified in both CD and UC genetics and pathogenesis of inflammatory bowel disease,[62], only few of them have been related to disease result. The presence of NOD2 polymorphism has been associated with a more serious clinical course of CD (that is, higher risk of intestinal strictures, earlier need for first surgery and lower postoperative disease-free interval).[63] Genetic factors associated with response to treatment have also been assessed in some studies.[61] There was also an association between polymorphisms in multi-drug resistant 1 (MDR1), TNF and migration inhibitory factor (MIF) genes, and corticosteroid refractoriness or sensitivity in CD and UC. In patients with steroid-resistant UC, MDR1 polymorphism was associated with an increased risk of cyclosporine failure. The induction of apoptosis in activated T lymphocytes partly generates the efficacy of infliximab. Infliximab-treated patients were examined by a research group from Leuven, who identified polymorphisms in apoptosis genes anticipating response to infliximab therapy in luminal and fistulizing Crohn's disease. Genetic polymorphisms were suggested as useful predictors of anti-TNF therapy responsiveness in paediatric IBD by other authors. [61]

V. REFERENCES


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