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TOPIC HIGHLIGHT

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Role of genetics in the diagnosis and prognosis of Crohn's disease

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Abstract

Considering epidemiological, genetic and immunological data, we can conclude that the inflammatory bowel diseases are heterogeneous disorders of multifactorial etiology in which hereditability and environment interact to produce the disease. It is probable that patients have a genetic predisposition for the development of the disease coupled with disturbances in immunoregulation. Several genes have been so far related to the diagnosis of Crohn's disease. Those genes are related to innate pattern recognition receptors, to epithelial barrier homeostasis and maintenance of epithelial barrier integrity, to autophagy and to lymphocyte differentiation. So far, the most strong and replicated associations with Crohn's disease have been done with *NOD2*, *IL23R* and *ATG16L1* genes. Many genes have so far been implicated in prognosis of Crohn's disease and many attempts have been made to classify genetic profiles in Crohn's disease. *CARD15* seems not only a susceptibility gene, but also a disease-modifier gene for Crohn's disease. Enriching our understanding on Crohn's disease genetics is important but when combining genetic data with functional data the outcome could be of major importance to clinicians.

Key words: Crohn's; Genetics; Polymorphism; Diagnosis; Prognosis; Genome wide scan; Genetic consortium

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EVOLVING ROLE OF GENETICS IN CROHN'S DISEASE

Despite decades of research the etiology of inflammatory bowel diseases (IBD) remains largely unexplained, but considering together epidemiological, genetic and immunological data, we can conclude that IBD are heterogeneous disorders of multifactorial etiology in which hereditability (genetic) and environment (microbial, behavior) interact to produce the immunological background of the disease. It is probable that patients have a genetic predisposition for the development of the disease coupled with disturbances in immunoregulation. The disease can then be triggered by any of a number of different unknown environmental factors and sustained by an abnormal immune response to these factors. Rather, the intensive interaction between intestinal epithelial cells and immune competent cells is critical to maintain and perpetuate the chronic inflammatory process characteristic

for IBD[1].

Early epidemiologic evidence for the role of genetic factors in the pathogenesis of Crohn's disease (CD) came from studies demonstrating higher rates of CD among individuals of Caucasian and Jewish ethnicity, familial aggregation of CD and higher concordance rates of both twins developing CD in monozygotic compared with dizygotic twins. The search for specific CD susceptibility genes, however, has been difficult due to complex genetics, including factors such as the lack of simple Mendelian inheritance patterns, involvement of several genes, and the influence of environmental factors and intestinal microflora on disease development. More than 30 distinct genomic loci encode genes involved in a number of homeostatic mechanisms and have been suggested to be involved in CD etiopathogenesis and prognosis[2].

Until very recently, two main approaches could be undertaken to identify genes in complex diseases: the positional cloning approach, based on linkage analysis, and the candidate gene approach, based on association studies. Linkage analysis studies the co-segregation of the disease with a marker within families. The candidate gene approach uses case-control cohorts or trios of affected offspring with both parents. Here, a specific gene with known or potential interest for the disease is studied. The allelic frequencies (in the case of case-control study) or the transmission of a single nucleotide polymorphism (SNP) towards affected offspring (in the case of trios) are analyzed, and differences between patients and controls, or distortion of transmission towards affected children, will point towards implication of the gene in the pathogenesis of the disease under investigation.

Despite the large numbers of genome-wide association studies (GWAS) established to date, most diseases have only managed to explain some additional percentage of the hereditability estimates. In an attempt to explain some of this missing hereditability, researchers have adopted several complementary strategies. Larger cohorts of cases are being collected, through either further patient recruitment or collaborations. The meta-analysis data generated to date has demonstrated how increasing the cohort sample size generates additional statistical power to detect smaller and smaller odds ratios[3]. Advances in technology and particularly bioinformatics have now made it possible to perform GWAS using common copy number variation probes. Many groups are looking to high-throughput sequencing technology, with the aim of sequencing candidate gene regions identified by GWAS, to hopefully identify either the causal or rare variants[4,5]. Several GWAS have been published in the last decade and have identified many genes associated with Crohn's disease (Table 1). Among these there are recognition-related genes such as *NOD1* and *TLRs*, other susceptibility genes including *DLG5*, *OCTN* and *HLA* and the newest susceptibility genes in CD resulting from GWAS: *IL23R* gene, *ATG16L1* gene and *IRGM* gene[6].

Table 1 Genetic polymorphisms related to Crohn's disease

Genes and the diagnosis of Crohn's disease
Genes related to innate pattern recognition receptors
NOD2/CARD15
OCTN
TLR
Genes related to epithelial barrier homeostasis
IBD5
DLG5
Genes related to molecular mimicry and autophagy
ATG16L1
IRGM
LRRK2
Genes related to lymphocyte differentiation
IL23R
STAT3
Genes related to secondary immune response and apoptosis
MHC
HLA
Genes and the prognosis of Crohn's disease
Genes related to age of Crohn's disease onset
TNFRSF6B, CXCL9, IL23R, NOD2 , ATG16L1, CNR1, IL-10, MDR1, DLG5, IRGM
Genes related to Crohn's disease behaviour
Stenotic/structuring behaviour: *NOD2, TLR4, IL-12B, CX3CR1, IL-10, IL-6*
Penetrating/fistulizing behaviour: *NOD2, IRGM, TNF, HLADRB1, CDKAL1*
Inflammatory behaviour: *HLA*
Granulomatous disease: *TLR4/CARD15*
Genes related to Crohn's disease location
Upper gastrointestinal: *NOD2, MIF*
Ileal: *IL-10, CRP, NOD2, ZNF365, STAT3*
Ileocolonic: *ATG16L1, TCF-4 (TCF7L2)*
Colonic: *HLA, TLR4, TLR1, -2, -6*
Genes related to Crohn's disease activity
HSP70-2, NOD2, PAI-1, CNR1
Genes related to surgery
NOD2 , HLA-G
Genes related to dysplasia and cancer
FHIT
Genes related to extraintestinal manifestations
*CARD15, FcRL3, HLADRB*103, HLAB*27 HLA-B*44, HLA-B*35, TNFa-308A, TNF-1031C, STAT3*
Pharmacogenetics in Crohn's disease
CARD15, NAT, TPMT, MDR1, MIF, DLG5, TNF, LTA

ROLE OF GENES IN THE DIAGNOSIS OF CROHN'S DISEASE

Several genes have been related to the diagnosis of Crohn's disease so far. Those genes are related to innate pattern recognition receptors, to epithelial barrier homeostasis and maintenance of epithelial barrier integrity, to autophagy and to lymphocyte differentiation. So far, the strongest and replicated associations with CD have been done with *NOD2*, *IL23R* and *ATG16L1* genes.

Genes related to innate pattern recognition receptors

***NOD2/CARD15* gene:** *NOD2*/Caspase Recruitment Domain Family member 15 (*CARD15*) acts as a pattern recognition receptor (PRR); this locus has been characterized as the IBD1 locus on 16q12-13[7].

Fine mapping of the IBD1 locus identified the underlying gene on chromosome 16 as the *CARD15* (previous *NOD2)* gene. *CARD15* represents homology with the R genes in plants, genes that confer resistance to infection[8]. Thirty nonconservative polymorphisms have been identified within the gene, which are associated with CD, but only three are common (Arg702Trp, Gly908Arg and Leuc1007insC). The three common variants account for approximately 82% of the mutated alleles. CARD15 is associated with CD only and not with UC. CARD15 codes for a protein expressed in monocytes, macrophages, dentritic cells, epithelial cells and Paneth cells. CARD15 is involved in the recognition of bacterial peptidoglycan-derived muramyl dipeptide through the leucine-rich repeat (LRR) region. Of importance, the frameshift mutation 1007fsinsC that leads to a truncated protein lacking the 33 distal amino acids was associated with impaired activation of the transcription factor NF-κB after stimulation.

It has been shown that Paneth cells play an important role in innate host defense via their ability to secrete antimicrobial peptides and proteins. Although NODs are expressed at low levels in absorptive and secretory intestinal epithelial cells, Paneth cells in the small intestine have been recognized as the predominant site of expression of NOD2 in the epithelium. Furthermore, NOD2 mutations have been associated with decreased expression of antimicrobial peptides, the α-defensins, by Paneth cells. In addition, a distinct gene polymorphism resulting in low β-defensin 2-gene copy number has been associated with a predisposition to colonic Crohn's disease. In addition, NOD2 plays important roles in the promotion of antibacterial T-helper-17 (Th-17) cells in the IL-23-IL-1-IL-17 axis.

CARD15 variants are found in 35% to 45% of white CD patients, with the exception of Scandinavian, Irish and Scottish patients[9,10], in whom the prevalence is much lower. Genotype relative risks of 3 (simple mutation) and 10-44 (double mutations) have been reported in European Caucasians[9,10]. However, CARD15 mutation is not frequent or even absent in African-American populations, in Indians, Chinese and Japanese[11-13]. Other CARD related genetic loci that have been associated with CD diagnosis are the CARD4 (NOD1), CARD8 and CARD9 loci[14,15].

Organic cation transporter genes: Organic cation transporters (*OCTNs, 5q31-33*) are membrane transporters for drugs and positively charged endogenous metabolites. The novel OCTN subfamily may also transport carnitine, which is essential for metabolism of lipids and is involved in transport of light chain fatty acids into mitochondria for b-oxidation. The first study reported on two functional mutations in the carnitine/OCTN cluster on 5q31 (the IBD5 locus) that were associated with Crohn's disease. As membrane transporters of organic cations, OCTNs are therefore important in the maintenance of intracellular homeostasis. In humans OCTN1 and OCTN2 map to IBD5 on 5q31. An OCTN3 has recently been described in humans[16].

Toll-like receptor genes: Host response to microbial pathogens includes self-defense mechanisms such as defensins, PRRs, pathogen-associated molecular patterns and toll-like receptors (TLRs). TLRs recognize conserved motifs on pathogens that are not found in higher eukaryotes and initiate an "innate" (rapid and non-specific) immune response[17]. Subsequently, specific receptors recognizing chemo-attractant molecules mobilize phagocytic leukocytes and induce their migration to inflammatory sites. There, leukocytes encounter the invading microorganisms and ingest them through the activation of phagocytic receptors that mediate the uptake process. Innate immune responses are linked to the generation of corresponding adaptive immune responses and studies of genetically engineered or cellularly manipulated animal models have generated a great deal of new information[18].

Leucocyte-epithelial interactions are of special interest as exposure of epithelial TLRs to microbial ligands has been shown to result in transcriptional upregulation of inflammatory mediators whereas ligation of leucocyte TLRs modulate specific antimicrobial responses[19]. It has been shown that Paneth cells play an important role in innate host defense *via* their ability to secrete antimicrobial peptides and proteins. In addition, it has been shown that NOD2 mutations lead to loss of negative regulatory effects on TLR signaling while activation of the CARD domain results in activation of NF-κB[20].

TLRs are the most important receptors of the innate immune system. They are expressed by immune cells and by intestinal epithelial cells in IBD patients. In humans, at least 10 different TLRs are described and each recognizes a specific pathogen-associated molecular pattern. A transmission disequilibrium test on Belgian IBD trios with CD demonstrated preferential transmission of the TLR4 Asp299Gly polymorphism from heterozygous parents to affected children[21]. TLR9 modulates CD susceptibility and there is interaction between other polymorphisms such as NOD2, IL23R and DLG5[22,23].

Genes related to epithelial barrier homeostasis

The gastrointestinal tract uses a system of tolerance and controlled inflammation to limit the response to dietary or bacteria-derived antigens in the gut[24]. When this complex system breaks down, either by a chemical or pathogenic insult in a genetically predisposed individual the resulting immune response may lead to IBD[25]. Genes or loci involved in the maintenance of epithelial barrier integrity and associated with Crohn's disease are the *IBD5* and the Discs Large Homolog 5 (*DLG5*)[26].

The *DLG5* gene is a 180-kb protein containing 1900 amino acids. DLG5 protein harbours a CARD domain, is a further CD susceptibility gene of the CARD family and contributes to CARD-mediated mechanisms of host defense. In fact, the *DLG5* gene associated protein is a member of Membrane Associated Guanylate Kinase family of scaffolding proteins. Scaffolding proteins

organize protein complexes at cellular junctions to integrate the tethering of adhesion molecules, receptors and intracellular signaling enzymes. Of interest is a population variation in DLG5 variants. For example, DLG5 R30Q variant was not confirmed in other European studies[27,28]. Other genes of potential importance in the same panel are the *PTGER4, ITLN1, DMBT1, BPI* and *XBP1* genes[29].

Genes related to molecular mimicry and autophagy

The innate immune system is the first line of defense against infection. Of interest, virulence factors from bacteria and viruses have been identified that manipulate host innate immune signaling pathways through molecular mimicry. These microbial proteins contain signaling domains that bear sequence and structural similarity to their host targets, and thereby potentially sabotage host immunity by hijacking crucial signaling pathways and uncouple receptor activation from effector induction. Several protein families have evolved to function as receptors or sensors of pathogen invasion. There are two types of signaling domains for the above receptors: the TIR domain for the TLRs and the Pyrin domain or CARD for the NOD-like receptors (NLRs) and retinoic acid-inducible gene 1-like receptors or helicases (RLRs or RLHs).

Molecular mimicry has been invoked as one of the mechanisms responsible for the activation of autoreactive cells by microbial peptides that have structural similarities to self peptides but there is also evidence that antigenically unrelated infections or specific inflammatory signals can result in autoaggressiveness and induction of organ-specific autoimmunity including the gut. The extent and severity of this loss of tolerance is still being defined, as it has demonstrated that loss of tolerance in IBD patients is not exclusive for bacterial antigens and occurs also to orally administered soluble proteins[30]. This subversion of innate immune signaling through molecular mimicry is closely related to the phenomenon of autophagy. Autophagy is the tightly orchestrated cellular 'housekeeping' process responsible for the degradation of damaged and dysfunctional cellular organelles and protein aggregates and is well recognized as playing an important role in maintaining cellular homeostasis under physiological and pathophysiological conditions. Regulated degradation and turnover of subcellular components is essential for normal cellular function, growth, and development. The major catabolic pathway responsible for the disposal of obsolete or damaged organelles and protein aggregates is autophagy (i.e., "self-digestion"). During this process organelles and proteins are encircled in a double-membrane vesicle (the autophagosome), delivered to lysosomes, and the substrates for ATP generation that can be recycled to synthesize new proteins, high-energy phosphates, and other cellular components. Autophagy has evolved as a conserved mechanism for cell survival under conditions of starvation and stress. In addition to (macro)autophagy, characterized by the sequestration of organelles and proteins within an autophagosome, there are two additional subtypes of self-digestion, microautophagy which is protrusion of the lysosomal membrane per se around a region of cytoplasm and chaperone-mediated autophagy in which degradation is restricted only to those proteins with a consensus peptide sequence recognized by specific chaperone complexes[31]. Autophagy is now considered to be important for host defense against intracellular microorganisms. The associations of these autophagy-associated genes with Crohn's disease strongly support the hypothesis that abnormal innate immune responses to intracellular pathogens contribute to the pathogenesis of Crohn's disease. In fact, the pathological characteristics of human Crohn's disease represent "granuloma" formation. The mechanisms of granuloma formation remain unclear. Recent studies have demonstrated functional roles for IL-23 in the differentiation and promotion of Th-17 cells. Autophagy genes that have been related to CD diagnosis are the *ATG16L1*[32,33], *IRGM* and the *LRRK2* gene[34]. Unraveling the mechanisms of such molecular mimicry is crucial to our understanding and clinical intervention of infectious diseases and inflammatory disorders of unkown aetiopathogenesis including Crohn's disease.

Genes related to lymphocyte differentiation

***IL23R* gene:** Dysregulated cytokine production by mucosal lymphocytes and macrophages has been implicated in the pathogenesis of CD. In fact, an exclusive increase of $CD4^+$ T cells in inflammatory bowel disease and their recruitment as intraepithelial lymphocytes has been demonstrated[35]. $CD4^+$ T cells secreting interleukin-17 (T helper type 17) cells have emerged as a key effector population driving colitis in animal models previously associated with exaggerated T helper type 1 responses.

Of the genes involved in the differentiation of Th-17 lymphocytes the *IL23R* gene has been proved of great importance and has been related to Crohn's disease[36,37].

The IL23R, consisting of an IL-12β1 and an IL23R chain, is highly expressed on memory T cells. IL23 is a novel cytokine formed *via* the binding of IL12p40 to a p19 protein. After binding to the IL23 receptor, IL23 preferentially activates memory T cells. IL23 does exhibit some similar biological activities to IL-12; however, IL-12 is more involved in the differentiation of naïve T-cells into Th1 lymphocytes and subsequent interferon-gamma production. IL23, on the other hand, mediates proinflammatory activities in part by the production of IL17 through activation of Th17 lymphocytes[38].

Signal transducer and activator of transcription 3 gene: Signal transducer and activator of transcription 3 (STAT3) play an important role in various autoimmune disorders including IBD[39,40]. STAT3 was initially identified as an acute phase response factor, an inducible DNA binding protein that binds to the IL-6 responsive element within the promoters of hepatic acute phase protein genes and is involved in IL-6 dependent T-cell proliferation through prevention of apoptosis. Subsequent studies indicate that STAT3 becomes activated in response to

a wide variety of cytokines and growth factors. Recent studies have revealed that STAT3 activation plays distinctly different roles between innate immune responses and acquired immune responses in colitis. STAT-3 mediated activation of acquired immune responses plays a pathogenic role in colitis by enhancing the survival of pathogenic T-cells. In contrast, STAT3-mediated activation of innate responses contributes to the suppression of colitis. Emerging data indicate that STAT3 is one of the crucial targets for the treatment of IBD. However, as the receptors of these cytokines and growth factors are present in both innate and acquired cells, activation of STAT3 is likely to occur in both cell types. Therefore as the function of STAT3 is a double-edged sword, careful attention should be directed toward the cell population that is being targeted when one contemplates STAT3 inhibition or activation in human IBD[41]. Within the same panel, other than *STAT3* genes, and with probable importance are the *TNFSF15, JAK2, CCR6* and *ICOSLG* genes[42-44].

Genes related to secondary immune response, apoptosis and other pathways

Chemokines play a central role in the pathogenesis of IBD as they are able to trigger multiple inflammatory actions including leukocyte activation and chemoattraction, granule exocytosis, production of metalloproteinases for matrix degradation and upregulation of the oxidative burst[45]. Therefore, further support is given for genes that relates to secondary immune response, apoptosis and other pathways. For example, in the IBD4 locus 4 several interesting candidate genes, which may be relevant in the pathogenesis of CD, lie within this region (e.g., genes regulating apoptosis, signal transduction proteins, chemokine receptors, T cell receptor, metalloproteinases).

Gene expression profiles from colon lamina propria fibroblasts have demonstrated several functional changes in some proteins coded from the corresponding genes: collagen types Ⅰ, Ⅳ, XIV, matrix metalloproteinase 1, cathepsin K, stroma cell-derived factor-1, chitinase3-like-1 and many others[46]. The major histocompatability complex (MHC) has been extensively investigated. Human leucocyte antigen (HLA) class Ⅱ molecules present partially digested antigen to the T-cell receptor and play a central role in the immune response. In CD MHC and HLA studies have yielded conflicting and heterogenous results. HLADR1 has been implicated with CD[47].

Many other genes, loci and chromosomes involved in CD have also been advocated in several studies that however still require wide replication and association with clinical practice. These include *CNR1, MCP-1*[48], *PTPN2* (protein tyrosine phosphatase)[49], *PTPN22, NKX-3, IL-18 RAP /IL-18R1, IL12/IL23* pathway[50], *PTGER4, MST1/BSN/MST1R*[50,51,52], *IL-2/IL-21*[53], *TYK2, JUN, NAT2*[54], *IL-10, NELL1, NKX2-3*[55], *Cyclin Y, Hect domain, 1q24, 10q21, 5p13, RCC1-like domain, ICOSLG, CDKAL1*[56], *13q13.3, 1p35.2, 3p29, 5p13.1*[57,58], *X chromosome*[59], *NLRP3*[60], *Vitamin D receptor polymorphisms*[61] and many others as well.

Genes in family and ethnic group studies

Linkage studies performed in complex genetic disorders such as CD frequently use model-free analytic methods, which are non-parametric analyses that do not assume Mendelian recessive or dominant models of inheritance.

The strongest risk factor for IBD is having a relative with the same disease. First-degree relatives of patients with CD have a 12-to-15 times greater risk of developing CD than do people of comparable age in the general population[61]. Familial clustering can also result from exposure to common environmental risk factors. Twin studies are very useful to determine the degree of genetic versus nongenetic etiologies for a trait. Today, there is no evidence of a separate entity of familial IBD[62,63]. Based on the current literature, phenotypic differences between familial and sporadic cases of IBD are weak. Available data are to be accepted with caution, however, as they are mostly retrospective and may be biased. CARD15 explains around 20% of the genetic predisposition to Crohn's disease[64]. The relative risk of developing CD in the presence of one mutation is 2-4, but increases dramatically in the case of two mutations (compound heterozygous or homozygous).

Although NOD2 provides no clear familial predisposition, unaffected relatives carry an increased rate of CARD15 variants (37.1%) compared to controls, and it would be interesting to see if they will eventually develop symptoms[65-67]. In addition, maternal transmission of CARD15 variants seems protective with a lower ratio of affected/unaffected children when compared to fathers[68,69]. In the light of the foregoing data, it seems that genetic counselling should be done with caution. In addition, families should not receive genetic counseling/information about age at onset and disease severity. Ethnic group studies and ethnic variation were first demonstrated in the Jewish population, and those studies are of major importance in this context[70].

ROLE OF GENES IN PROGNOSIS OF CROHN'S DISEASE

This is a major issue that greatly concerns patients. Many genes have so far been implicated in the prognosis of CD and numerous attempts have been made to classify the genetic profiles in CD. Of interest, CARD15 seems not only a susceptibility gene, but also a disease-modifier gene for CD. Of the many studies published on the clinical relevance of CARD15 mutations, there are several providing data on disease location, and the majority of them support a significant association of CARD15 mutations with ileal disease site, while some demonstrate a connection with the absence of colonic location. Some studies also provide data supporting the relevance to CARD15 variants with stricturing disease behavior, and also penetrating behaviour. Other pertinent studies revealed an association with early onset of the disease. These investigations also support the thesis that pediatric Crohn's is like a "more genetic disease" consistent with other polygenic disease

models. Other reports provide data on an increased risk or need of surgery related to CD[71].

Differences among studies are difficult to explain, and we could argue about the low number of patients in some of the studies, the disease variability among Caucasians and finally differences regarding disease assessment and interobserver agreement. Whether the described relationship between the CARD15 variants and both stenosing phenotype and increased need for surgery in CD patients is a true association or only reflects the high proportion of ileal CD developing bowel stenosis and, therefore, requiring surgery, is still a matter of controversy.

Genes related to age of Crohn's disease onset

With respect to age of CD onset and more specially to childhood or early-onset Crohn's disease, many genes/loci have been implicated: *TNFRSF6B, CXCL9*[72], *IL23R*[73,74], *NOD2*[75], *ATG16L1 rs2241880*[76], *CNR1*[77], *IL-10*[78], *MDR1*[79]. Of interest DLG5 seems protective for female children[80] while there are also studies not supporting the relation of genes and early onset of CD[81] or supporting the relation of IL-10 and IRGM with adult onset[82].

Genes related to crohn's disease behaviour

Genes related to stenotic/structuring behaviour in CD are: *NOD2/CARD15*[83], *TLR4*[84], *IL-12B*[85], and *CX-3CR1*[86,87]. Of importance NOD2/CARD15 has been also related to acute intestinal obstruction[88]. IL-10 and IL-6 are also potentially related to stenotic/structuring behaviour in CD while genetic variants of several metalloproteinases and their inhibitors would be excellent candidate genes, since these molecules are considered to play a key role in the abnormal fibrogenesis that underlies the development of bowel stenosis in CD patients. Genes related to penetrating/fistulizing behaviour in CD are as follows: *NOD2, IRGM, TNF*[89], *HLADRB1*[90]; the C-allele in CDKAL1 rs6908425 SNP is associated with NOD2 (-) perianal fistula, whereas OCTN and the near *IL-12B* gene rs12704036 T-allele have a relationship with non perianal fistula[91]. Inflammatory CD behaviour has been related to HLA variation[92] while granulomatous disease has been related with TLR4/CARD15 variants[93].

Genes related to Crohn's disease location

Upper gastrointestinal Crohn's disease has been related to NOD2[94] and MIF variants[95]. Ileal CD has been related to the following genes: *IL-10*[96], *CRP* gene[97], *NOD2, ZNF365* and *STAT3*[98]. Genes/loci associated with ileocolonic CD are *3p21*, *ATG16L1*[98] and *TCF-4* (*TCF7L2*)[99]. No role for phenotype in IL23R gene has been demonstrated[100] while a detailed genotype-phenotype analysis revealed weak associations of the IL23R rs10024819 variant with ileal involvement and stenoses in carriers of the TT genotype. Finally, the HLADRB1*0701 has been associated with ileal CD, but only in patients that have no CARD15 variants[101]. Colonic CD has been related to the following genes: the HLA region was associated with inflammatory colonic phenotype and TLR4[102], TLR1, -2, -6[103]. *TNF* gene showed a negative association with stricturing behaviour or colonic location[104]. For IBD5 and OCTN1 and 2, results have not been consistent but associations with perianal and ileal disease have been reported.

Genes related to Crohn's disease activity

Genes implicated in disease activity are the following: *HSP70-2* heat shock protein gene[105], *NOD2*[106], *PAI-1* (type 1 plasminogen activator inhibitor[107]), while the combination of NOD2 and PAI-1 predicted complicated disease behavior[108]. Of importance, NOD2 predicted lower weight in children[109], and CNR1 low BMI[110].

Genes related to surgery

NOD2 gene has been related to early pediatric surgery[111], stenosis and need for surgery[112], previous surgeries[113], increased number of surgeries[107] and surgical costs[114]. NOD2 has no relation to the risk of re-operation[115]. Finally, HLA-G has been associated with higher risk for ileocolonic resection[116].

Genes related to dysplasia and cancer

The *FHIT* gene (fragile histidine triad gene) located at 3p14.2 has been identified as a candidate tumor-suppressor gene. The gene spans the t (3; 8) translocation breakpoint of familial renal-cell carcinoma and contains the FRA3B fragile site. It encodes the human diadenosine triphosphate hydrolase, which in vitro cleaves the diadenosine substrate into ADP and AMP. It has been suggested that *FHIT* gene plays a role in the pathogenesis of IBD and the development and progression of a subgroup of IBD-related carcinomas at an early phase[117-119].

Genes related to extraintestinal manifestations and concommitant diseases

Extraintestinal manifestations are common in CD. Genes related to CD extraintestinal manifestations have been reported, as follows. Peripheral arthritis was related with FcRL3[120], HLADRB*103, HLAB*27 HLA-B*44, HLA-B*35, TNFalpha-308A[121]. CARD15 has been related to spondyloarthropathy[122] and uveitis[123] but not with sacroileitis[124]. TNF-1031C was associated with erythema nodosum while certain HLA alleles (HLA-B27, HLA-B35, HLA-B44) were connected with different disease behaviour and extraintestinal manifestations such as arthropathy, eye and skin manifestations. Genes/loci related to other chronic diseases concomitant to CD are 10p12.2 (sarcoidosis and CD)[125], STAT3 (multiple sclerosis and CD)[126], and a parallel genetic fingerprint between leprosy and CD[127].

Pharmacogenetics in Crohn's disease

Pharmacogenetics is of major importance in CD therapeutics and prognosis. Genes have been implicated in influencing the efficacy and side effects of drugs and reflect a complex interplay regarding absorption, elimina-

tion and transport. Future studies need to be large and prospective with uniformly phenotyped patients and correlating genetic associations with functional data. In addition hypotheses such as whether observations about drug response in IBD lead us to IBD etiology or whether the genes that control the drug response are related to genes that control the disease still remain unanswered. Pharmacogenetic studies to date have found no association between CARD15 variants and prediction of response to various IBD therapies. In addition, responses to azathioprine, steroids and infliximab are not related to NOD2[128]. Of note, NOD2 was related to antibiotic failure[129].For mesalazine, variability in drug acetylation was demonstrated many years ago with patients divided in slow and rapid acetylators, because of polymorphisms in the N-acetyltransferase (*NAT*) genes. Two isoenzymes NAT1 and NAT2 have been identified in humans and more than 50% of Caucasians are NAT2 slow acetylators. Mesalazine is acetylated in the liver by NAT1 into N-acetyl-5 aminosalicylates and excreted in the urine[47].

The clinical usefulness of pharmacogenetics in CD is limited to AZA and TPMT at this moment. The human TPMT gene, consisting of 10 exons, is located on chromosome 6p22.3. The hereditary nature of the TPMT deficiency in humans was initially identified in a study of TPMT activity in red blood cells (RBC). This and subsequent studies determined the distribution of TPMT activity in RBC to be trimodal; 90% of persons have high activity, 10% have intermediate activity and 0.3% have low or no detectable enzyme activity. To date, numerous mutant TPMT alleles have been identified, including the three most frequent alleles (TPMT*2, TPMT*3A and TPMT*3C), which account for 80%-95% of intermediate or low TPMT enzyme activity cases. The prevalence of the most frequent SNPs in the TPMT gene has been reported to vary worldwide. However, it is of interest that studies on the prevalence of TPMT SNPs in large IBD cohorts are lacking. Although AZA is an effective drug for maintenance of remission in IBD, it is associated with side effects. Clinically sound pharmacogenetic studies over the last two decades have shown that polymorphisms in the *TPMT* gene locus play a significant role in the occurrence of various side effects of thiopurine drugs including life-threatening bone marrow toxicity (BMT), a serious dose-related toxicity[130-134].

The G2677T variant in the *MDR1* gene predicted gastrointestinal and unspecified intolerance to azathioprine and methotrexate in IBD patients. These findings suggest a role for MDR1/P-gp in the mechanism of action of azathioprine and methotrexate[135,136].

Twin studies have linked polymorhisms of the vitamin D receptor (*VDR*) gene with bone mineral density in healthy women and in addition VDR is an important regulator of calcium metabolism and bone cell function and influences calcium absorption from the intestine. VDR polymorphisms have also been implicated in susceptibility to CD[137].

The HLADR region has been associated with failure to budesonide[138] while DLG5R30Q predicted response to steroids[139]. Other genes such as MIF (macrophage migration inhibition)[140] and MDR have been also related to steroid therapy[136]. In addition, 1082 AA IL-10 genotype was associated with steroid dependency, whereas the allele 113A of the *DLG5* gene conferred resistance to steroids.

Regarding response to infliximab the data for TNF gene are conflicting. Specifically, there are conflicting data regarding the role of FcGR3A, which has been supported by some authors[141,142], but was not confirmed in patients of the ACCENT I study. Response to infliximab is not related to *TNFa-308*[143] or *TNFR1* and *TNFR2*[144] or *NOD*[145] or *CRP* gene[136]. The association between the Fas ligand-843 TT genotype and lack of response to infliximab seemed to be the most relevant observation[136]. The relationship of infliximab response and lymphotoxin alpha gene (*LTA*) is also conflicting[144].

WHAT LIES AHEAD

Gene-to-gene crosstalk and epistasis

With new methodologies like genome wide association studies, microarrays, and fine SNP analysis becoming available during the last decade, our investigative armamentarium has been considerably enriched. As many studies with complex statistics arise, we understand increasingly the real crosstalk present among genes and the need of a genetic panel for disease diagnosis and prognosis. It is now evident that gene-to-gene interaction and epistasis modulate disease activity and susceptibility[146]. Some data have come to light. A genome-wide scan in a Flemish population of IBD affected families supports the existence of *IBD4* on 14q11, and has shown additional evidence for the existence of other susceptibility loci (1p, 4q and 10p). This study has further demonstrated that epistasis and gene to gene interactions (CARD15-TLR4) are also present in IBD and that population heterogeneity is not to be underestimated[147]. Crosstalk has been demonstrated for TLR9 with NOD2, IL23R and DLG5, and epistasis has been shown between IL23R and DLG5. Also potential epistasis between IL23R variants and the three other previously described CD susceptibility genes CARD15, SLC22A4 and SLC22A5 (OCTN 1 and 2) has been shown[116].

Genetic consortium studies and genome wide scans

Over the past few years, a combination of progress in high throughput genotyping technology and growing knowledge about the human genome through the International HapMap project and the Human Genome Project have enabled genome-wide association studies (GWAS) for several complex diseases. To understand the approach to conducting GWAS in this setting it is important to expound on the concept of linkage disequilibrium, which refers to the nonrandom association of alleles at nearby loci. Specifically, linkage disequilibrium refers to adjacent alleles assorting together nonindepen-

Table 2 Predicted future developments in the genetics of Crohn's disease

What lies ahead in the genetics of Crohn's disease
Gene-to-gene crosstalk and epistasis
Genome wide association studies
Microarrays
Fine single nucleotide polymorphism analysis
Genetic consortium studies and genome wide scans
Genome-wide association studies
Genetic consortium studies
Future perspectives
Functional studies to understand the mechanisms
Combining genetic data with functional data
Combination of a panel of clinical, biochemical, serological and genetic factors
Functional consequences of polymorphisms
Molecular and cellular mechanisms leading to Crohn's disease
Predict disease outcomes
Redesigning the methods of treatment

dently from generation to generation because they are tightly linked and thus less likely to become separated by recombination. Genetic consortium studies are of major importance and homogeneity in methodology issues is of paramount value[148-151]. Appropriate study design[152], power analysis[153] and overall data analysis and meta-analysis[154] are mandatory. Accurate estimation of sample sizes required in a genetic association study is essential before commencing genotyping, to ensure that the study is sufficiently powered to detect the subtle genetic effects that contribute to most complex diseases. The extensive genetic variation and complex linkage disequilibrium across even a small genomic region will give rise to several alternative scenarios. Genetic variation across a region studied should be carefully evaluated and consideration should be given to possible linkage disequilibrium and allelic heterogeneity when evaluating power of an association study. As larger datasets are studied and combined, as genotyping platforms provide even greater depth of coverage of the genome and as modest hits are followed up in large independent panels so that the vast majority of true signals should be identified. These robust genetic data will truly provide a solid platform for functional studies to understand the mechanisms by which these genetic variants predispose to CD. Finally studies at post-transcriptional level become more and more urgent[155]. Enriching our understanding of CD genetics is important but when combining genetic data with functional data the outcome could be of major importance. In fact, improved understanding of immune mechanisms, on which manifold genetic and environmental traits might converge, and which ultimately mediate all phenomena in inflammatory bowel disease, holds promise (Table 2).

CONCLUSION

The recent advances in the understanding of CD genetics have been tremendous[156]. Starting with the susceptibility area, whole genome linkage and association scans have already led to the identification of a number of susceptibility genes (*NOD2/CARD15, DLG5, OCTN1* and *2, NOD1, IL23R, PTGER4, ATG16L1* and *IRGM*) of which the *NOD2/CARD15* gene is the most replicated and understood at present. Although it is clear that genetic research in IBD has advanced our understanding of the clinical heterogeneity of the disease, new efforts are required and point towards the complex combination of a panel of clinical, biochemical, serological and genetic factors, in order to achieve the optimal prediction of both clinical behaviour and response to therapy.

Genome-wide association studies have allowed an unprecedented rapid unraveling of the genetic basis of IBD; however there will be much more follow-up work needed in this field. First, ongoing work including meta-analysis of the Crohn's disease genome wide association studies will probably reveal additional Crohn's disease susceptibility genes. It will then be essential to investigate the functional consequences of polymorphisms in these genes so the molecular and cellular mechanisms leading to CD can be better characterized. Finally, genotype-phenotype correlation studies should help clinicians predict disease outcomes with more accuracy, including the risk for complications, need for surgery, and response to therapy, and finally lead to redesigning the methods of treatment of CD patients.

REFERENCES

1 **Achkar JP**, Duerr R. The expanding universe of inflammatory bowel disease genetics. *Curr Opin Gastroenterol* 2008; **24**: 429-434
2 **Van Limbergen J**, Wilson DC, Satsangi J. The genetics of Crohn's disease. *Annu Rev Genomics Hum Genet* 2009; **10**: 89-116
3 **van Heel DA**, Ghosh S, Butler M, Hunt KA, Lundberg AM, Ahmad T, McGovern DP, Onnie C, Negoro K, Goldthorpe S, Foxwell BM, Mathew CG, Forbes A, Jewell DP, Playford RJ. Muramyl dipeptide and toll-like receptor sensitivity in NOD2-associated Crohn's disease. *Lancet* 2005; **365**: 1794-1796
4 **Yamamoto S**, Ma X. Role of Nod2 in the development of Crohn's disease. *Microbes Infect* 2009; **11**: 912-918
5 **Colombel JF**. The CARD15 (also known as NOD2) gene in Crohn's disease: are there implications for current clinical practice? *Clin Gastroenterol Hepatol* 2003; **1**: 5-9
6 **Henckaerts L**, Van Steen K, Verstreken I, Cleynen I, Franke A, Schreiber S, Rutgeerts P, Vermeire S. Genetic risk profiling and prediction of disease course in Crohn's disease patients. *Clin Gastroenterol Hepatol* 2009; **7**: 972-980.e2
7 **Hugot JP**, Laurent-Puig P, Gower-Rousseau C, Olson JM, Lee JC, Beaugerie L, Naom I, Dupas JL, Van Gossum A, Orholm M, Bonaiti-Pellie C, Weissenbach J, Mathew CG, Lennard-Jones JE, Cortot A, Colombel JF, Thomas G. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. *Nature* 1996; **379**: 821-823
8 **Vermeire S**. Review article: genetic susceptibility and application of genetic testing in clinical management of inflammatory bowel disease. *Aliment Pharmacol Ther* 2006; **24** Suppl 3: 2-10
9 **Boedeker EC**. Gut microbes, the innate immune system and inflammatory bowel disease: location, location, location. *Curr Opin Gastroenterol* 2007; **23**: 1-3
10 **Vermeire S**. DLG5 and OCTN. *Inflamm Bowel Dis* 2004; **10**: 888-890

11 **Pugazhendhi S**, Amte A, Balamurugan R, Subramanian V, Ramakrishna BS. Common NOD2 mutations are absent in patients with Crohn's disease in India. *Indian J Gastroenterol* 2008; **27**: 201-203
12 **Leong RW**, Armuzzi A, Ahmad T, Wong ML, Tse P, Jewell DP, Sung JJ. NOD2/CARD15 gene polymorphisms and Crohn's disease in the Chinese population. *Aliment Pharmacol Ther* 2003; **17**: 1465-1470
13 **Inoue N**, Tamura K, Kinouchi Y, Fukuda Y, Takahashi S, Ogura Y, Inohara N, Núñez G, Kishi Y, Koike Y, Shimosegawa T, Shimoyama T, Hibi T. Lack of common NOD2 variants in Japanese patients with Crohn's disease. *Gastroenterology* 2002; **123**: 86-91
14 **Molnar T**, Hofner P, Nagy F, Lakatos PL, Fischer S, Lakatos L, Kovacs A, Altorjay I, Papp M, Palatka K, Demeter P, Tulassay Z, Nyari T, Miheller P, Papp J, Mandi Y, Lonovics J. NOD1 gene E266K polymorphism is associated with disease susceptibility but not with disease phenotype or NOD2/CARD15 in Hungarian patients with Crohn's disease. *Dig Liver Dis* 2007; **39**: 1064-1070
15 **Browning BL**, Annese V, Barclay ML, Bingham SA, Brand S, Büning C, Castro M, Cucchiara S, Dallapiccola B, Drummond H, Ferguson LR, Ferraris A, Fisher SA, Gearry RB, Glas J, Henckaerts L, Huebner C, Knafelz D, Lakatos L, Lakatos PL, Latiano A, Liu X, Mathew C, Müller-Myhsok B, Newman WG, Nimmo ER, Noble CL, Palmieri O, Parkes M, Petermann I, Rutgeerts P, Satsangi J, Shelling AN, Siminovitch KA, Török HP, Tremelling M, Vermeire S, Valvano MR, Witt H. Gender-stratified analysis of DLG5 R30Q in 4707 patients with Crohn disease and 4973 controls from 12 Caucasian cohorts. *J Med Genet* 2008; **45**: 36-42
16 **Newman B**, Gu X, Wintle R, Cescon D, Yazdanpanah M, Liu X, Peltekova V, Van Oene M, Amos CI, Siminovitch KA. A risk haplotype in the Solute Carrier Family 22A4/22A5 gene cluster influences phenotypic expression of Crohn's disease. *Gastroenterology* 2005; **128**: 260-269
17 **De Jager PL**, Franchimont D, Waliszewska A, Bitton A, Cohen A, Langelier D, Belaiche J, Vermeire S, Farwell L, Goris A, Libioulle C, Jani N, Dassopoulos T, Bromfield GP, Dubois B, Cho JH, Brant SR, Duerr RH, Yang H, Rotter JI, Silverberg MS, Steinhart AH, Daly MJ, Podolsky DK, Louis E, Hafler DA, Rioux JD. The role of the Toll receptor pathway in susceptibility to inflammatory bowel diseases. *Genes Immun* 2007; **8**: 387-397
18 **Bokoch GM**. Regulation of innate immunity by Rho GTPases. *Trends Cell Biol* 2005; **15**: 163-171
19 **Liew FY**, Xu D, Brint EK, O'Neill LA. Negative regulation of toll-like receptor-mediated immune responses. *Nat Rev Immunol* 2005; **5**: 446-458
20 **Watanabe T**, Kitani A, Strober W. NOD2 regulation of Toll-like receptor responses and the pathogenesis of Crohn's disease. *Gut* 2005; **54**: 1515-1518
21 **Pierik M**, Joossens S, Van Steen K, Van Schuerbeek N, Vlietinck R, Rutgeerts P, Vermeire S. Toll-like receptor-1, -2, and -6 polymorphisms influence disease extension in inflammatory bowel diseases. *Inflamm Bowel Dis* 2006; **12**: 1-8
22 **Cucchiara S**, Latiano A, Palmieri O, Staiano AM, D'Incà R, Guariso G, Vieni G, Rutigliano V, Borrelli O, Valvano MR, Annese V. Role of CARD15, DLG5 and OCTN genes polymorphisms in children with inflammatory bowel diseases. *World J Gastroenterol* 2007; **13**: 1221-1229
23 **Török HP**, Glas J, Tonenchi L, Lohse P, Müller-Myhsok B, Limbersky O, Neugebauer C, Schnitzler F, Seiderer J, Tillack C, Brand S, Brünnler G, Jagiello P, Epplen JT, Griga T, Klein W, Schiemann U, Folwaczny M, Ochsenkühn T, Folwaczny C. Polymorphisms in the DLG5 and OCTN cation transporter genes in Crohn's disease. *Gut* 2005; **54**: 1421-1427
24 **Geboes K**. From inflammation to lesion. *Acta Gastroenterol Belg* 1994; **57**: 273-284
25 **Sartor RB**. Pathogenesis and immune mechanisms of chronic inflammatory bowel diseases. *Am J Gastroenterol* 1997; **92**: 5S-11S
26 **Achkar JP**, Fiocchi C. Gene-gene interactions in inflammatory bowel disease: biological and clinical implications. *Am J Gastroenterol* 2009; **104**: 1734-1736
27 **Friedrichs F**, Henckaerts L, Vermeire S, Kucharzik T, Seehafer T, Möller-Krull M, Bornberg-Bauer E, Stoll M, Weiner J. The Crohn's disease susceptibility gene DLG5 as a member of the CARD interaction network. *J Mol Med* (Berl) 2008; **86**: 423-432
28 **Vermeire S**, Pierik M, Hlavaty T, Claessens G, van Schuerbeeck N, Joossens S, Ferrante M, Henckaerts L, Bueno de Mesquita M, Vlietinck R, Rutgeerts P. Association of organic cation transporter risk haplotype with perianal penetrating Crohn's disease but not with susceptibility to IBD. *Gastroenterology* 2005; **129**: 1845-1853
29 **Klein W**, Tromm A, Folwaczny C, Hagedorn M, Duerig N, Epplen JT, Schmiegel WH, Griga T. A polymorphism of the NFKBIA gene is associated with Crohn's disease patients lacking a predisposing allele of the CARD15 gene. *Int J Colorectal Dis* 2004; **19**: 153-156
30 **Xiao TS.** Subversion of innate immune signaling through molecular mimicry. *J Clin Immunol* 2010; **30**: 638-642
31 **Dong Y,** Undyala VV, Gottlieb RA, Mentzer RM, Przyklenk K. Autophagy: definition, molecular machinery and potential role in myocardial ischemia-reperfusion injury. *J Cardiovasc Pharmacol* 2010 July 1; Epub ahead of print
32 **Rioux JD**, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A, Green T, Kuballa P, Barmada MM, Datta LW, Shugart YY, Griffiths AM, Targan SR, Ippoliti AF, Bernard EJ, Mei L, Nicolae DL, Regueiro M, Schumm LP, Steinhart AH, Rotter JI, Duerr RH, Cho JH, Daly MJ, Brant SR. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat Genet* 2007; **39**: 596-604
33 **Hampe J**, Franke A, Rosenstiel P, Till A, Teuber M, Huse K, Albrecht M, Mayr G, De La Vega FM, Briggs J, Günther S, Prescott NJ, Onnie CM, Häsler R, Sipos B, Fölsch UR, Lengauer T, Platzer M, Mathew CG, Krawczak M, Schreiber S. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nat Genet* 2007; **39**: 207-211
34 **McCarroll SA**, Huett A, Kuballa P, Chilewski SD, Landry A, Goyette P, Zody MC, Hall JL, Brant SR, Cho JH, Duerr RH, Silverberg MS, Taylor KD, Rioux JD, Altshuler D, Daly MJ, Xavier RJ. Deletion polymorphism upstream of IRGM associated with altered IRGM expression and Crohn's disease. *Nat Genet* 2008; **40**: 1107-1112
35 **Kaser A**, Blumberg RS. Adaptive immunity in inflammatory bowel disease: state of the art. *Curr Opin Gastroenterol* 2008; **24**: 455-461
36 **Brand S**. Crohn's disease: Th1, Th17 or both? The change of a paradigm: new immunological and genetic insights implicate Th17 cells in the pathogenesis of Crohn's disease. *Gut* 2009; **58**: 1152-1167
37 **Duerr RH**, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, Abraham C, Regueiro M, Griffiths A, Dassopoulos T, Bitton A, Yang H, Targan S, Datta LW, Kistner EO, Schumm LP, Lee AT, Gregersen PK, Barmada MM, Rotter JI, Nicolae DL, Cho JH. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 2006; **314**: 1461-1463
38 **Dubinsky MC**, Wang D, Picornell Y, Wrobel I, Katzir L, Quiros A, Dutridge D, Wahbeh G, Silber G, Bahar R, Mengesha E, Targan SR, Taylor KD, Rotter JI. IL-23 receptor (IL-23R) gene protects against pediatric Crohn's disease. *Inflamm Bowel Dis* 2007; **13**: 511-515
39 **Amre DK**, Mack DR, Morgan K, Israel D, Deslandres C, Seidman EG, Lambrette P, Costea I, Krupoves A, Fegury H, Dong J, Grimard G, Levy E. Susceptibility loci reported in

genome-wide association studies are associated with Crohn's disease in Canadian children. *Aliment Pharmacol Ther* 2010; **31**: 1186-1191

40 **Sato K**, Shiota M, Fukuda S, Iwamoto E, Machida H, Inamine T, Kondo S, Yanagihara K, Isomoto H, Mizuta Y, Kohno S, Tsukamoto K. Strong evidence of a combination polymorphism of the tyrosine kinase 2 gene and the signal transducer and activator of transcription 3 gene as a DNA-based biomarker for susceptibility to Crohn's disease in the Japanese population. *J Clin Immunol* 2009; **29**: 815-825

41 **Sugimoto K**. Role of STAT3 in inflammatory bowel disease. *World J Gastroenterol* 2008; **14**: 5110-5114

42 **Yamazaki K**, Takahashi A, Takazoe M, Kubo M, Onouchi Y, Fujino A, Kamatani N, Nakamura Y, Hata A. Positive association of genetic variants in the upstream region of NKX2-3 with Crohn's disease in Japanese patients. *Gut* 2009; **58**: 228-232

43 **Tremelling M**, Parkes M. Genome-wide association scans identify multiple confirmed susceptibility loci for Crohn's disease: lessons for study design. *Inflamm Bowel Dis* 2007; **13**: 1554-1560

44 **Lees CW**, Satsangi J. Genetics of inflammatory bowel disease: implications for disease pathogenesis and natural history. *Expert Rev Gastroenterol Hepatol* 2009; **3**: 513-534

45 **Danese S**, Gasbarrini A. Chemokines in inflammatory bowel disease. *J Clin Pathol* 2005; **58**: 1025-1027

46 **Lang M**, Schlechtweg M, Kellermeier S, Brenmoehl J, Falk W, Schölmerich J, Herfarth H, Rogler G, Hausmann M. Gene expression profiles of mucosal fibroblasts from strictured and nonstrictured areas of patients with Crohn's disease. *Inflamm Bowel Dis* 2009; **15**: 212-223

47 **Henckaerts L**, Figueroa C, Vermeire S, Sans M. The role of genetics in inflammatory bowel disease. *Curr Drug Targets* 2008; **9**: 361-368

48 **Palmieri O**, Latiano A, Salvatori E, Valvano MR, Bossa F, Latiano T, Corritore G, di Mauro L, Andriulli A, Annesec V. The -A2518G polymorphism of monocyte chemoattractant protein-1 is associated with Crohn's disease. *Am J Gastroenterol* 2010; **105**: 1586-1594

49 **Amre DK**, Mack DR, Morgan K, Fujiwara M, Israel D, Deslandres C, Seidman EG, Lambrette P, Costea I, Krupoves A, Fegury H, Dong J, Grimard G, Levy E. Investigation of reported associations between the 20q13 and 21q22 loci and pediatric-onset Crohn's disease in Canadian children. *Am J Gastroenterol* 2009; **104**: 2824-2828

50 **Wang K**, Zhang H, Kugathasan S, Annese V, Bradfield JP, Russell RK, Sleiman PM, Imielinski M, Glessner J, Hou C, Wilson DC, Walters T, Kim C, Frackelton EC, Lionetti P, Barabino A, Van Limbergen J, Guthery S, Denson L, Piccoli D, Li M, Dubinsky M, Silverberg M, Griffiths A, Grant SF, Satsangi J, Baldassano R, Hakonarson H. Diverse genome-wide association studies associate the IL12/IL23 pathway with Crohn Disease. *Am J Hum Genet* 2009; **84**: 399-405

51 **Russell RK**, Nimmo ER, Satsangi J. Molecular genetics of Crohn's disease. *Curr Opin Genet Dev* 2004; **14**: 264-270

52 **Beckly JB**, Hancock L, Geremia A, Cummings JR, Morris A, Cooney R, Pathan S, Guo C, Jewell DP. Two-stage candidate gene study of chromosome 3p demonstrates an association between nonsynonymous variants in the MST1R gene and Crohn's disease. *Inflamm Bowel Dis* 2008; **14**: 500-507

53 **Márquez A**, Varadé J, Robledo G, Martínez A, Mendoza JL, Taxonera C, Fernández-Arquero M, Díaz-Rubio M, Gómez-García M, López-Nevot MA, de la Concha EG, Martín J, Urcelay E. Specific association of a CLEC16A/KIAA0350 polymorphism with NOD2/CARD15(-) Crohn's disease patients. *Eur J Hum Genet* 2009; **17**: 1304-1308

54 **Machida H**, Tsukamoto K, Wen CY, Shikuwa S, Isomoto H, Mizuta Y, Takeshima F, Murase K, Matsumoto N, Murata I, Kohno S, Wen CY. Crohn's disease in Japanese is associated with a SNP-haplotype of N-acetyltransferase 2 gene. *World J Gastroenterol* 2005; **11**: 4833-4837

55 **Weersma RK**, Stokkers PC, Cleynen I, Wolfkamp SC, Henckaerts L, Schreiber S, Dijkstra G, Franke A, Nolte IM, Rutgeerts P, Wijmenga C, Vermeire S. Confirmation of multiple Crohn's disease susceptibility loci in a large Dutch-Belgian cohort. *Am J Gastroenterol* 2009; **104**: 630-638

56 **Barrett JC**, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, Brant SR, Silverberg MS, Taylor KD, Barmada MM, Bitton A, Dassopoulos T, Datta LW, Green T, Griffiths AM, Kistner EO, Murtha MT, Regueiro MD, Rotter JI, Schumm LP, Steinhart AH, Targan SR, Xavier RJ, Libioulle C, Sandor C, Lathrop M, Belaiche J, Dewit O, Gut I, Heath S, Laukens D, Mni M, Rutgeerts P, Van Gossum A, Zelenika D, Franchimont D, Hugot JP, de Vos M, Vermeire S, Louis E, Cardon LR, Anderson CA, Drummond H, Nimmo E, Ahmad T, Prescott NJ, Onnie CM, Fisher SA, Marchini J, Ghori J, Bumpstead S, Gwilliam R, Tremelling M, Deloukas P, Mansfield J, Jewell D, Satsangi J, Mathew CG, Parkes M, Georges M, Daly MJ. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet* 2008; **40**: 955-962

57 **Libioulle C**, Louis E, Hansoul S, Sandor C, Farnir F, Franchimont D, Vermeire S, Dewit O, de Vos M, Dixon A, Demarche B, Gut I, Heath S, Foglio M, Liang L, Laukens D, Mni M, Zelenika D, Van Gossum A, Rutgeerts P, Belaiche J, Lathrop M, Georges M. Novel Crohn disease locus identified by genome-wide association maps to a gene desert on 5p13.1 and modulates expression of PTGER4. *PLoS Genet* 2007; **3**: e58

58 **Urcelay E**, Mendoza JL, Martín MC, Mas A, Martínez A, Taxonera C, Fernandez-Arquero M, Díaz-Rubio M, de la Concha EG. MDR1 gene: susceptibility in Spanish Crohn's disease and ulcerative colitis patients. *Inflamm Bowel Dis* 2006; **12**: 33-37

59 **Vermeire S**, Satsangi J, Peeters M, Parkes M, Jewell DP, Vlietinck R, Rutgeerts P. Evidence for inflammatory bowel disease of a susceptibility locus on the X chromosome. *Gastroenterology* 2001; **120**: 834-840

60 **Villani AC**, Lemire M, Fortin G, Louis E, Silverberg MS, Collette C, Baba N, Libioulle C, Belaiche J, Bitton A, Gaudet D, Cohen A, Langelier D, Fortin PR, Wither JE, Sarfati M, Rutgeerts P, Rioux JD, Vermeire S, Hudson TJ, Franchimont D. Common variants in the NLRP3 region contribute to Crohn's disease susceptibility. *Nat Genet* 2009; **41**: 71-76

61 **Simmons JD**, Mullighan C, Welsh KI, Jewell DP. Vitamin D receptor gene polymorphism: association with Crohn's disease susceptibility. *Gut* 2000; **47**: 211-214

62 **Peeters M**, Cortot A, Vermeire S, Colombel JF. Familial and sporadic inflammatory bowel disease: different entities? *Inflamm Bowel Dis* 2000; **6**: 314-320

63 **Brant SR**, Shugart YY. Inflammatory bowel disease gene hunting by linkage analysis: rationale, methodology, and present status of the field. *Inflamm Bowel Dis* 2004; **10**: 300-311

64 **Vignal C**, Singer E, Peyrin-Biroulet L, Desreumaux P, Chamaillard M. How NOD2 mutations predispose to Crohn's disease? *Microbes Infect* 2007; **9**: 658-663

65 **Akolkar PN**, Gulwani-Akolkar B, Heresbach D, Lin XY, Fisher S, Katz S, Silver J. Differences in risk of Crohn's disease in offspring of mothers and fathers with inflammatory bowel disease. *Am J Gastroenterol* 1997; **92**: 2241-2244

66 **Vermeire S**, Van Assche G, Rutgeerts P. Should family members of IBD patients be screened for CARD15/NOD2 mutations? *Inflamm Bowel Dis* 2008; **14** Suppl 2: S190-S191

67 **Csillag C**, Nielsen OH, Borup R, Olsen J, Bjerrum JT, Nielsen FC. CARD15 status and familial predisposition for Crohn's disease and colonic gene expression. *Dig Dis Sci* 2007; **52**: 1783-1789

68 **Esters N**, Pierik M, van Steen K, Vermeire S, Claessens G, Joossens S, Vlietinck R, Rutgeerts P. Transmission of

CARD15 (NOD2) variants within families of patients with inflammatory bowel disease. *Am J Gastroenterol* 2004; **99**: 299-305
69 **Heliö T**, Halme L, Lappalainen M, Fodstad H, Paavola-Sakki P, Turunen U, Färkkilä M, Krusius T, Kontula K. CARD15/NOD2 gene variants are associated with familially occurring and complicated forms of Crohn's disease. *Gut* 2003; **52**: 558-562
70 **Shugart YY**, Silverberg MS, Duerr RH, Taylor KD, Wang MH, Zarfas K, Schumm LP, Bromfield G, Steinhart AH, Griffiths AM, Kane SV, Barmada MM, Rotter JI, Mei L, Bernstein CN, Bayless TM, Langelier D, Cohen A, Bitton A, Rioux JD, Cho JH, Brant SR. An SNP linkage scan identifies significant Crohn's disease loci on chromosomes 13q13.3 and, in Jewish families, on 1p35.2 and 3q29. *Genes Immun* 2008; **9**: 161-167
71 **Radford-Smith G**, Pandeya N. Associations between NOD2/CARD15 genotype and phenotype in Crohn's disease--Are we there yet? *World J Gastroenterol* 2006; **12**: 7097-7103
72 **Lacher M**, Schroepf S, Ballauff A, Lohse P, von Schweinitz D, Kappler R, Koletzko S. Autophagy 16-like 1 rs2241880 G allele is associated with Crohn's disease in German children. *Acta Paediatr* 2009; **98**: 1835-1840
73 **Baldassano RN**, Bradfield JP, Monos DS, Kim CE, Glessner JT, Casalunovo T, Frackelton EC, Otieno FG, Kanterakis S, Shaner JL, Smith RM, Eckert AW, Robinson LJ, Onyiah CC, Abrams DJ, Chiavacci RM, Skraban R, Devoto M, Grant SF, Hakonarson H. Association of variants of the interleukin-23 receptor gene with susceptibility to pediatric Crohn's disease. *Clin Gastroenterol Hepatol* 2007; **5**: 972-976
74 **Amre DK**, Mack DR, Morgan K, Krupoves A, Costea I, Lambrette P, Grimard G, Dong J, Feguery H, Bucionis V, Deslandres C, Levy E, Seidman EG. Autophagy gene ATG16L1 but not IRGM is associated with Crohn's disease in Canadian children. *Inflamm Bowel Dis* 2009; **15**: 501-507
75 **Gazouli M**, Pachoula I, Panayotou I, Mantzaris G, Chrousos G, Anagnou NP, Roma-Giannikou E. NOD2/CARD15, ATG16L1 and IL23R gene polymorphisms and childhood-onset of Crohn's disease. *World J Gastroenterol* 2010; **16**: 1753-1758
76 **Amre DK**, Mack DR, Morgan K, Israel D, Lambrette P, Costea I, Krupoves A, Fegury H, Dong J, Grimard G, Deslandres C, Levy E, Seidman EG. Interleukin 10 (IL-10) gene variants and susceptibility for paediatric onset Crohn's disease. *Aliment Pharmacol Ther* 2009; **29**: 1025-1031
77 **Storr M**, Emmerdinger D, Diegelmann J, Pfennig S, Ochsenkühn T, Göke B, Lohse P, Brand S. The cannabinoid 1 receptor (CNR1) 1359 G/A polymorphism modulates susceptibility to ulcerative colitis and the phenotype in Crohn's disease. *PLoS One* 2010; **5**: e9453
78 **Sanchez R**, Levy E, Costea F, Sinnett D. IL-10 and TNF-alpha promoter haplotypes are associated with childhood Crohn's disease location. *World J Gastroenterol* 2009; **15**: 3776-3782
79 **Krupoves A**, Seidman EG, Mack D, Israel D, Morgan K, Lambrette P, Costea I, Deslandres C, Grimard G, Law L, Levy E, Amre DK. Associations between ABCB1/MDR1 gene polymorphisms and Crohn's disease: a gene-wide study in a pediatric population. *Inflamm Bowel Dis* 2009; **15**: 900-908
80 **Biank V**, Friedrichs F, Babusukumar U, Wang T, Stoll M, Broeckel U, Kugathasan S. DLG5 R30Q variant is a female-specific protective factor in pediatric onset Crohn's disease. *Am J Gastroenterol* 2007; **102**: 391-398
81 **Essers JB**, Lee JJ, Kugathasan S, Stevens CR, Grand RJ, Daly MJ. Established genetic risk factors do not distinguish early and later onset Crohn's disease. *Inflamm Bowel Dis* 2009; **15**: 1508-1514
82 **Latiano A**, Palmieri O, Cucchiara S, Castro M, D'Incà R, Guariso G, Dallapiccola B, Valvano MR, Latiano T, Andriulli A, Annese V. Polymorphism of the IRGM gene might predispose to fistulizing behavior in Crohn's disease. *Am J Gastroenterol* 2009; **104**: 110-116
83 **Barreiro-de Acosta M**, Peña AS. Clinical applications of NOD2/CARD15 mutations in Crohn's disease. *Acta Gastroenterol Latinoam* 2007; **37**: 49-54
84 **Brand S**, Staudinger T, Schnitzler F, Pfennig S, Hofbauer K, Dambacher J, Seiderer J, Tillack C, Konrad A, Crispin A, Göke B, Lohse P, Ochsenkühn T. The role of Toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms and CARD15/NOD2 mutations in the susceptibility and phenotype of Crohn's disease. *Inflamm Bowel Dis* 2005; **11**: 645-652
85 **Aithal GP**, Day CP, Leathart J, Daly AK, Hudson M. Association of single nucleotide polymorphisms in the interleukin-4 gene and interleukin-4 receptor gene with Crohn's disease in a British population. *Genes Immun* 2001; **2**: 44-47
86 **Sabate JM**, Ameziane N, Lamoril J, Jouet P, Farmachidi JP, Soulé JC, Harnois F, Sobhani I, Jian R, Deybach JC, de Prost D, Coffin B. The V249I polymorphism of the CX3CR1 gene is associated with fibrostenotic disease behavior in patients with Crohn's disease. *Eur J Gastroenterol Hepatol* 2008; **20**: 748-755
87 **Brand S**, Beigel F, Olszak T, Zitzmann K, Eichhorst ST, Otte JM, Diepolder H, Marquardt A, Jagla W, Popp A, Leclair S, Herrmann K, Seiderer J, Ochsenkühn T, Göke B, Auernhammer CJ, Dambacher J. IL-22 is increased in active Crohn's disease and promotes proinflammatory gene expression and intestinal epithelial cell migration. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G827-G838
88 **Guagnozzi D**, Cossu A, Viscido A, Corleto V, Annese V, Latiano A, Delle Fave G, Caprilli R. Acute intestinal obstruction and NOD2/CARD15 mutations among Italian Crohn's disease patients. *Eur Rev Med Pharmacol Sci* 2004; **8**: 179-185
89 **González S**, Rodrigo L, Martínez-Borra J, López-Vázquez A, Fuentes D, Niño P, Cadahía V, Saro C, Dieguez MA, López-Larrea C. TNF-alpha -308A promoter polymorphism is associated with enhanced TNF-alpha production and inflammatory activity in Crohn's patients with fistulizing disease. *Am J Gastroenterol* 2003; **98**: 1101-1106
90 **Bouma G**, Poen AC, García-González MA, Schreuder GM, Felt-Bersma RJ, Meuwissen SG, Pena AS. HLA-DRB1*03, but not the TNFA -308 promoter gene polymorphism, confers protection against fistulising Crohn's disease. *Immunogenetics* 1998; **47**: 451-455
91 **Vermeire S**, Pierik M, Hlavaty T, Claessens G, van Schuerbeeck N, Joossens S, Ferrante M, Henckaerts L, Bueno de Mesquita M, Vlietinck R, Rutgeerts P. Association of organic cation transporter risk haplotype with perianal penetrating Crohn's disease but not with susceptibility to IBD. *Gastroenterology* 2005; **129**: 1845-1853
92 **Burton PR**, Clayton DG, Cardon LR, Craddock N, Deloukas P, Duncanson A, Kwiatkowski DP, McCarthy MI, Ouwehand WH, Samani NJ, Todd JA, Donnelly P, Barrett JC, Burton PR, Davison D, Donnelly P, Easton D, Evans D, Leung HT, Marchini JL, Morris AP, Spencer CC, Tobin MD, Cardon LR, Clayton DG, Attwood AP, Boorman JP, Cant B, Everson U, Hussey JM, Jolley JD, Knight AS, Koch K, Meech E, Nutland S, Prowse CV, Stevens HE, Taylor NC, Walters GR, Walker NM, Watkins NA, Winzer T, Todd JA, Ouwehand WH, Jones RW, McArdle WL, Ring SM, Strachan DP, Pembrey M, Breen G, St Clair D, Caesar S, Gordon-Smith K, Jones L, Fraser C, Green EK, Grozeva D, Hamshere ML, Holmans PA, Jones IR, Kirov G, Moskvina V, Nikolov I, O'Donovan MC, Owen MJ, Craddock N, Collier DA, Elkin A, Farmer A, Williamson R, McGuffin P, Young AH, Ferrier IN, Ball SG, Balmforth AJ, Barrett JH, Bishop DT, Iles MM, Maqbool A, Yuldasheva N, Hall AS, Braund PS, Burton PR, Dixon RJ, Mangino M, Suzanne S, Tobin MD, Thompson JR, Samani NJ, Bredin F, Tremelling M, Parkes M, Drummond H, Lees CW, Nimmo ER, Satsangi J, Fisher SA, Forbes A,

Lewis CM, Onnie CM, Prescott NJ, Sanderson J, Mathew CG, Barbour J, Mohiuddin MK, Todhunter CE, Mansfield JC, Ahmad T, Cummings FR, Jewell DP, Webster J, Brown MJ, Clayton DG, Lathrop GM, Connell J, Dominczak A, Samani NJ, Marcano CA, Burke B, Dobson R, Gungadoo J, Lee KL, Munroe PB, Newhouse SJ, Onipinla A, Wallace C, Xue M, Caulfield M, Farrall M, Barton A, Bruce IN, Donovan H, Eyre S, Gilbert PD, Hider SL, Hinks AM, John SL, Potter C, Silman AJ, Symmmons DP, Thomson W, Worthington J, Clayton DG, Dunger DB, Nutland S, Stevens HE, Walker NM, Widmer B, Todd JA, Frayling TA, Freathy RM, Lango H, Perry JR, Shields BM, Weedon MN, Hattersley AT, Hitman GA, Walker M, Elliott KS, Groves CJ, Lindgren CM, Rayner NW, Timpson NJ, Zeggini E, McCarthy MI, Newport M, Sirugo G, Lyons E, Vanberg F, Hill AV, Bradbury LA, Farrar C, Pointon JJ, Wordsworth P, Brown MA, Franklyn JA, Heward JM, Simmonds MJ, Gough SC, Seal S, Stratton MR, Rahman N, Ban M, Goris A, Sawcer SJ, Compston A, Conway D, Jallow M, Newport M, Sirugo G, Rockett KA, Kwiatowski DP, Bumpstead SJ, Chaney A, Downes K, Ghori MJ, Gwilliam R, Hunt SE, Inouye M, Keniry A, King E, McGinnis R, Potter S, Ravindrarajah R, Whittaker P, Widden C, Withers D, Deloukas P, Leung HT, Nutland S, Stevens HE, Walker NM, Todd JA, Easton D, Clayton DG, Burton PR, Tobin MD, Barrett JC, Evans D, Morris AP, Cardon LR, Cardin NJ, Davison D, Ferreira T, Pereira-Gale J, Hallgrimsdottir IB, Howie BN, Marchini JL, Spencer CC, Su Z, Teo YY, Vukcevic D, Donnelly P, Bentley D, Brown MA, Gordon LR, Caulfield M, Clayton DG, Compston A, Craddock N, Deloukas P, Donnelly P, Farrall M, Gough SC, Hall AS, Hattersley AT, Hill AV, Kwiatkowski DP, Mathew C, McCarthy MI, Ouwehand WH, Parkes M, Pembrey M, Rahman N, Samani NJ, Stratton MR, Todd JA, Worthington J. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007; **447**: 661-678

93 **Pierik M**, De Hertogh G, Vermeire S, Van Assche G, Van Eyken P, Joossens S, Claessens G, Vlietinck R, Rutgeerts P, Geboes K. Epithelioid granulomas, pattern recognition receptors, and phenotypes of Crohn's disease. *Gut* 2005; **54**: 223-227

94 **Mardini HE**, Gregory KJ, Nasser M, Selby L, Arsenescu R, Winter TA, de Villiers WJ. Gastroduodenal Crohn's disease is associated with NOD2/CARD15 gene polymorphisms, particularly L1007P homozygosity. *Dig Dis Sci* 2005; **50**: 2316-2322

95 **Dambacher J**, Staudinger T, Seiderer J, Sisic Z, Schnitzler F, Pfennig S, Hofbauer K, Konrad A, Tillack C, Otte JM, Diebold J, Göke B, Ochsenkühn T, Lohse P, Brand S. Macrophage migration inhibitory factor (MIF) -173G/C promoter polymorphism influences upper gastrointestinal tract involvement and disease activity in patients with Crohn's disease. *Inflamm Bowel Dis* 2007; **13**: 71-82

96 **Amre DK**, Mack DR, Morgan K, Israel D, Lambrette P, Costea I, Krupoves A, Fegury H, Dong J, Grimard G, Deslandres C, Levy E, Seidman EG. Interleukin 10 (IL-10) gene variants and susceptibility for paediatric onset Crohn's disease. *Aliment Pharmacol Ther* 2009; **29**: 1025-1031

97 **Thalmaier D**, Dambacher J, Seiderer J, Konrad A, Schachinger V, Pfennig S, Otte JM, Crispin A, Göke B, Ochsenkühn T, Lohse P, Brand S. The +1059G/C polymorphism in the C-reactive protein (CRP) gene is associated with involvement of the terminal ileum and decreased serum CRP levels in patients with Crohn's disease. *Aliment Pharmacol Ther* 2006; **24**: 1105-1115

98 **Prescott NJ**, Fisher SA, Franke A, Hampe J, Onnie CM, Soars D, Bagnall R, Mirza MM, Sanderson J, Forbes A, Mansfield JC, Lewis CM, Schreiber S, Mathew CG. A nonsynonymous SNP in ATG16L1 predisposes to ileal Crohn's disease and is independent of CARD15 and IBD5. *Gastroenterology* 2007; **132**: 1665-1671

99 **Koslowski MJ**, Kübler I, Chamaillard M, Schaeffeler E, Reinisch W, Wang G, Beisner J, Teml A, Peyrin-Biroulet L, Winter S, Herrlinger KR, Rutgeerts P, Vermeire S, Cooney R, Fellermann K, Jewell D, Bevins CL, Schwab M, Stange EF, Wehkamp J. Genetic variants of Wnt transcription factor TCF-4 (TCF7L2) putative promoter region are associated with small intestinal Crohn's disease. *PLoS One* 2009; **4**: e4496

100 **Cummings JR**, Ahmad T, Geremia A, Beckly J, Cooney R, Hancock L, Pathan S, Guo C, Cardon LR, Jewell DP. Contribution of the novel inflammatory bowel disease gene IL23R to disease susceptibility and phenotype. *Inflamm Bowel Dis* 2007; **13**: 1063-1068

101 **Glas J**, Seiderer J, Wetzke M, Konrad A, Török HP, Schmechel S, Tonenchi L, Grassl C, Dambacher J, Pfennig S, Maier K, Griga T, Klein W, Epplen JT, Schiemann U, Folwaczny C, Lohse P, Göke B, Ochsenkühn T, Müller-Myhsok B, Folwaczny M, Mussack T, Brand S. rs1004819 is the main disease-associated IL23R variant in German Crohn's disease patients: combined analysis of IL23R, CARD15, and OCTN1/2 variants. *PLoS One* 2007; **2**: e819

102 **Ouburg S**, Mallant-Hent R, Crusius JB, van Bodegraven AA, Mulder CJ, Linskens R, Peña AS, Morré SA. The toll-like receptor 4 (TLR4) Asp299Gly polymorphism is associated with colonic localisation of Crohn's disease without a major role for the Saccharomyces cerevisiae mannan-LBP-CD14-TLR4 pathway. *Gut* 2005; **54**: 439-440

103 **Pierik M**, Joossens S, Van Steen K, Van Schuerbeek N, Vlietinck R, Rutgeerts P, Vermeire S. Toll-like receptor-1, -2, and -6 polymorphisms influence disease extension in inflammatory bowel diseases. *Inflamm Bowel Dis* 2006; **12**: 1-8

104 **Waschke KA**, Villani AC, Vermeire S, Dufresne L, Chen TC, Bitton A, Cohen A, Thomson AB, Wild GE. Tumor necrosis factor receptor gene polymorphisms in Crohn's disease: association with clinical phenotypes. *Am J Gastroenterol* 2005; **100**: 1126-1133

105 **Nam SY**, Kim N, Kim JS, Lim SH, Jung HC, Song IS. Heat shock protein gene 70-2 polymorphism is differentially associated with the clinical phenotypes of ulcerative colitis and Crohn's disease. *J Gastroenterol Hepatol* 2007; **22**: 1032-1038

106 **Klausz G**, Molnár T, Nagy F, Gyulai Z, Boda K, Lonovics J, Mándi Y. Polymorphism of the heat-shock protein gene Hsp70-2, but not polymorphisms of the IL-10 and CD14 genes, is associated with the outcome of Crohn's disease. *Scand J Gastroenterol* 2005; **40**: 1197-1204

107 **Alvarez-Lobos M**, Arostegui JI, Sans M, Tassies D, Piu J, Reverter JC, Pique JM, Yagüe J, Panés J. Combined type-1 plasminogen activator inhibitor and NOD2/CARD15 genotyping predicts complicated Crohn's disease behaviour. *Aliment Pharmacol Ther* 2007; **25**: 429-440

108 **Sans M**, Tàssies D, Pellisé M, Espinosa G, Quintó L, Piqué JM, Reverter JC, Panés J. The 4G/4G genotype of the 4G/5G polymorphism of the type-1 plasminogen activator inhibitor (PAI-1) gene is a determinant of penetrating behaviour in patients with Crohn's disease. *Aliment Pharmacol Ther* 2003; **17**: 1039-1047

109 **Tomer G**, Ceballos C, Concepcion E, Benkov KJ. NOD2/CARD15 variants are associated with lower weight at diagnosis in children with Crohn's disease. *Am J Gastroenterol* 2003; **98**: 2479-2484

110 **Storr M**, Emmerdinger D, Diegelmann J, Pfennig S, Ochsenkühn T, Göke B, Lohse P, Brand S. The cannabinoid 1 receptor (CNR1) 1359 G/A polymorphism modulates susceptibility to ulcerative colitis and the phenotype in Crohn's disease. *PLoS One* 2010; **5**: e9453

111 **Kugathasan S**, Collins N, Maresso K, Hoffmann RG, Stephens M, Werlin SL, Rudolph C, Broeckel U. CARD15 gene mutations and risk for early surgery in pediatric-onset Crohn's disease. *Clin Gastroenterol Hepatol* 2004; **2**: 1003-1009

112 **Seiderer J**, Elben I, Diegelmann J, Glas J, Stallhofer J, Tillack

C, Pfennig S, Jürgens M, Schmechel S, Konrad A, Göke B, Ochsenkühn T, Müller-Myhsok B, Lohse P, Brand S. Role of the novel Th17 cytokine IL-17F in inflammatory bowel disease (IBD): upregulated colonic IL-17F expression in active Crohn's disease and analysis of the IL17F p.His161Arg polymorphism in IBD. *Inflamm Bowel Dis* 2008; **14**: 437-445

113 **Barreiro M**, Núñez C, Domínguez-Muñoz JE, Lorenzo A, Barreiro F, Potel J, Peña AS. Association of NOD2/CARD15 mutations with previous surgical procedures in Crohn's disease. *Rev Esp Enferm Dig* 2005; **97**: 547-553

114 **Odes S**, Friger M, Vardi H, Claessens G, Bossuyt X, Riis L, Munkholm P, Wolters F, Yona H, Hoie O, Beltrami M, Tsianos E, Katsanos K, Mouzas I, Clofent J, Monteiro E, Messori A, Politi P, O'Morain C, Limonard C, Russel M, Vatn M, Moum B, Stockbrugger R, Vermeire S. Role of ASCA and the NOD2/CARD15 mutation Gly908Arg in predicting increased surgical costs in Crohn's disease patients: a project of the European Collaborative Study Group on Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2007; **13**: 874-881

115 **Maconi G**, Colombo E, Sampietro GM, Lamboglia F, D' Incà R, Daperno M, Cassinotti A, Sturniolo GC, Ardizzone S, Duca P, Porro GB, Annese V. CARD15 gene variants and risk of reoperation in Crohn's disease patients. *Am J Gastroenterol* 2009; **104**: 2483-2491

116 **Glas J**, Seiderer J, Wetzke M, Konrad A, Török HP, Schmechel S, Tonenchi L, Grassl C, Dambacher J, Pfennig S, Maier K, Griga T, Klein W, Epplen JT, Schiemann U, Folwaczny C, Lohse P, Göke B, Ochsenkühn T, Müller-Myhsok B, Folwaczny M, Mussack T, Brand S. rs1004819 is the main disease-associated IL23R variant in German Crohn's disease patients: combined analysis of IL23R, CARD15, and OCTN1/2 variants. *PLoS One* 2007; **2**: e819

117 **Ohta M**, Inoue H, Cotticelli MG, Kastury K, Baffa R, Palazzo J, Siprashvili Z, Mori M, McCue P, Druck T, Croce CM, Huebner K. The FHIT gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma-associated t(3; 8) breakpoint, is abnormal in digestive tract cancers. *Cell* 1996; **84**: 587-597

118 **Paradee W**, Mullins C, He Z, Glover T, Wilke C, Opalka B, Schutte J, Smith DI. Precise localization of aphidicolin-induced breakpoints on the short arm of human chromosome 3. *Genomics* 1995; **27**: 358-361

119 **Barnes LD**, Garrison PN, Siprashvili Z, Guranowski A, Robinson AK, Ingram SW, Croce CM, Ohta M, Huebner K. Fhit, a putative tumor suppressor in humans, is a dinucleoside 5′,5‴-P1,P3-triphosphate hydrolase. *Biochemistry* 1996; **35**: 11529-11535

120 **Mendoza JL**, Lana R, Martin MC, de la Concha EG, Urcelay E, Diaz-Rubio M, Abreu MT, Mitchell AA. FcRL3 gene promoter variant is associated with peripheral arthritis in Crohn's disease. *Inflamm Bowel Dis* 2009; **15**: 1351-1357

121 **González S**, Rodrigo L, Martínez-Borra J, López-Vázquez A, Fuentes D, Niño P, Cadahía V, Saro C, Dieguez MA, López-Larrea C. TNF-alpha -308A promoter polymorphism is associated with enhanced TNF-alpha production and inflammatory activity in Crohn's patients with fistulizing disease. *Am J Gastroenterol* 2003; **98**: 1101-1106

122 **Laukens D**, Peeters H, Marichal D, Vander Cruyssen B, Mielants H, Elewaut D, Demetter P, Cuvelier C, Van Den Berghe M, Rottiers P, Veys EM, Remaut E, Steidler L, De Keyser F, De Vos M. CARD15 gene polymorphisms in patients with spondyloarthropathies identify a specific phenotype previously related to Crohn's disease. *Ann Rheum Dis* 2005; **64**: 930-935

123 **Rodríguez-Pérez N**, Aguinaga-Barrilero A, Gorroño-Echebarría MB, Pérez-Blas M, Martín-Villa JM. Analysis of Crohn's disease-related CARD15 polymorphisms in Spanish patients with idiopathic uveitis. *Dis Markers* 2008; **24**: 111-117

124 **Peeters H**, Vander Cruyssen B, Mielants H, de Vlam K, Vermeire S, Louis E, Rutgeerts P, Belaiche J, De Vos M. Clinical and genetic factors associated with sacroiliitis in Crohn's disease. *J Gastroenterol Hepatol* 2008; **23**: 132-137

125 **Franke A**, Fischer A, Nothnagel M, Becker C, Grabe N, Till A, Lu T, Müller-Quernheim J, Wittig M, Hermann A, Balschun T, Hofmann S, Niemiec R, Schulz S, Hampe J, Nikolaus S, Nürnberg P, Krawczak M, Schürmann M, Rosenstiel P, Nebel A, Schreiber S. Genome-wide association analysis in sarcoidosis and Crohn's disease unravels a common susceptibility locus on 10p12.2. *Gastroenterology* 2008; **135**: 1207-1215

126 **Jakkula E**, Leppä V, Sulonen AM, Varilo T, Kallio S, Kemppinen A, Purcell S, Koivisto K, Tienari P, Sumelahti ML, Elovaara I, Pirttilä T, Reunanen M, Aromaa A, Oturai AB, Søndergaard HB, Harbo HF, Mero IL, Gabriel SB, Mirel DB, Hauser SL, Kappos L, Polman C, De Jager PL, Hafler DA, Daly MJ, Palotie A, Saarela J, Peltonen L. Genome-wide association study in a high-risk isolate for multiple sclerosis reveals associated variants in STAT3 gene. *Am J Hum Genet* 2010; **86**: 285-291

127 **Schurr E**, Gros P. A common genetic fingerprint in leprosy and Crohn's disease? *N Engl J Med* 2009; **361**: 2666-2668

128 **Weiss B**, Lebowitz O, Fidder HH, Maza I, Levine A, Shaoul R, Reif S, Bujanover Y, Karban A. Response to medical treatment in patients with Crohn's disease: the role of NOD2/CRAD15, disease phenotype, and age of diagnosis. *Dig Dis Sci* 2010; **55**: 1674-1680

129 **Angelberger S**, Reinisch W, Dejaco C, Miehsler W, Waldhoer T, Wehkamp J, Lichtenberger C, Schaeffeler E, Vogelsang H, Schwab M, Teml A. NOD2/CARD15 gene variants are linked to failure of antibiotic treatment in perianal fistulating Crohn's disease. *Am J Gastroenterol* 2008; **103**: 1197-1202

130 **Schwab M**, Schäffeler E, Marx C, Fischer C, Lang T, Behrens C, Gregor M, Eichelbaum M, Zanger UM, Kaskas BA. Azathioprine therapy and adverse drug reactions in patients with inflammatory bowel disease: impact of thiopurine S-methyltransferase polymorphism. *Pharmacogenetics* 2002; **12**: 429-436

131 **Srimartpirom S**, Tassaneeyakul W, Kukongviriyapan V, Tassaneeyakul W. Thiopurine S-methyltransferase genetic polymorphism in the Thai population. *Br J Clin Pharmacol* 2004; **58**: 66-70

132 **McLeod HL**, Pritchard SC, Githang'a J, Indalo A, Ameyaw MM, Powrie RH, Booth L, Collie-Duguid ES. Ethnic differences in thiopurine methyltransferase pharmacogenetics: evidence for allele specificity in Caucasian and Kenyan individuals. *Pharmacogenetics* 1999; **9**: 773-776

133 **Schwab M**, Schäffeler E, Marx C, Fischer C, Lang T, Behrens C, Gregor M, Eichelbaum M, Zanger UM, Kaskas BA. Azathioprine therapy and adverse drug reactions in patients with inflammatory bowel disease: impact of thiopurine S-methyltransferase polymorphism. *Pharmacogenetics* 2002; **12**: 429-436

134 **Lennard L**. TPMT in the treatment of Crohn's disease with azathioprine. *Gut* 2002; **51**: 143-146

135 **Bohanec Grabar P,** Logar D, Lestan B, Dolzan V. Genetic determinants of methotrexate toxicity in rheumatoid arthritis patients: a study of polymorphisms affecting methotrexate transport and folate metabolism. *Eur J Clin Pharmacol* 2008; **64**: 1057-1068

136 **Marzolini C**, Paus E, Buclin T, Kim RB. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin Pharmacol Ther* 2004; **75**: 13-33

137 **Todhunter CE**, Sutherland-Craggs A, Bartram SA, Donaldson PT, Daly AK, Francis RM, Mansfield JC, Thompson NP. Influence of IL-6, COL1A1, and VDR gene polymorphisms on bone mineral density in Crohn's disease. *Gut* 2005; **54**: 1579-1584

138 **Gelbmann CM**, Rogler G, Gierend M, Gross V, Schölmerich J, Andus T. Association of HLA-DR genotypes and IL-1ra

gene polymorphism with treatment failure of budesonide and disease patterns in Crohn's disease. *Eur J Gastroenterol Hepatol* 2001; **13**: 1431-1437
139 **Lakatos PL**, Szamosi T, Szilvasi A, Molnar E, Lakatos L, Kovacs A, Molnar T, Altorjay I, Papp M, Tulassay Z, Miheller P, Papp J, Tordai A, Andrikovics H. ATG16L1 and IL23 receptor (IL23R) genes are associated with disease susceptibility in Hungarian CD patients. *Dig Liver Dis* 2008; **40**: 867-873
140 **Griga T**, Wilkens C, Wirkus N, Epplen J, Schmiegel W, Klein W. A polymorphism in the macrophage migration inhibitory factor gene is involved in the genetic predisposition of Crohn's disease and associated with cumulative steroid doses. *Hepatogastroenterology* 2007; **54**: 784-786
141 **Louis E**, El Ghoul Z, Vermeire S, Dall'Ozzo S, Rutgeerts P, Paintaud G, Belaiche J, De Vos M, Van Gossum A, Colombel JF, Watier H. Association between polymorphism in IgG Fc receptor IIIa coding gene and biological response to infliximab in Crohn's disease. *Aliment Pharmacol Ther* 2004; **19**: 511-519
142 **Willot S**, Vermeire S, Ohresser M, Rutgeerts P, Paintaud G, Belaiche J, De Vos M, Van Gossum A, Franchimont D, Colombel JF, Watier H, Louis E. No association between C-reactive protein gene polymorphisms and decrease of C-reactive protein serum concentration after infliximab treatment in Crohn's disease. *Pharmacogenet Genomics* 2006; **16**: 37-42
143 **Louis E**, Vermeire S, Rutgeerts P, De Vos M, Van Gossum A, Pescatore P, Fiasse R, Pelckmans P, Reynaert H, D'Haens G, Malaise M, Belaiche J. A positive response to infliximab in Crohn disease: association with a higher systemic inflammation before treatment but not with -308 TNF gene polymorphism. *Scand J Gastroenterol* 2002; **37**: 818-824
144 **Pierik M**, Vermeire S, Steen KV, Joossens S, Claessens G, Vlietinck R, Rutgeerts P. Tumour necrosis factor-alpha receptor 1 and 2 polymorphisms in inflammatory bowel disease and their association with response to infliximab. *Aliment Pharmacol Ther* 2004; **20**: 303-310
145 **Barreiro-de Acosta M**, Peña AS. Clinical applications of NOD2/CARD15 mutations in Crohn's disease. *Acta Gastroenterol Latinoam* 2007; **37**: 49-54
146 **Emily M**, Mailund T, Hein J, Schauser L, Schierup MH. Using biological networks to search for interacting loci in genome-wide association studies. *Eur J Hum Genet* 2009; **17**: 1231-1240
147 **Vermeire S**, Rutgeerts P, Van Steen K, Joossens S, Claessens G, Pierik M, Peeters M, Vlietinck R. Genome wide scan in a Flemish inflammatory bowel disease population: support for the IBD4 locus, population heterogeneity, and epistasis. *Gut* 2004; **53**: 980-986
148 **Lettre G**, Rioux JD. Autoimmune diseases: insights from genome-wide association studies. *Hum Mol Genet* 2008; **17**: R116-R121
149 **Satsangi J**, Jewell D, Parkes M, Bell J. Genetics of inflammatory bowel disease. A personal view on progress and prospects. *Dig Dis* 1998; **16**: 370-374
150 **Raelson JV**, Little RD, Ruether A, Fournier H, Paquin B, Van Eerdewegh P, Bradley WE, Croteau P, Nguyen-Huu Q, Segal J, Debrus S, Allard R, Rosenstiel P, Franke A, Jacobs G, Nikolaus S, Vidal JM, Szego P, Laplante N, Clark HF, Paulussen RJ, Hooper JW, Keith TP, Belouchi A, Schreiber S. Genome-wide association study for Crohn's disease in the Quebec Founder Population identifies multiple validated disease loci. *Proc Natl Acad Sci USA* 2007; **104**: 14747-14752
151 **Mathew CG**. New links to the pathogenesis of Crohn disease provided by genome-wide association scans. *Nat Rev Genet* 2008; **9**: 9-14
152 **Tremelling M**, Parkes M. Genome-wide association scans identify multiple confirmed susceptibility loci for Crohn's disease: lessons for study design. *Inflamm Bowel Dis* 2007; **13**: 1554-1560
153 **Fisher SA**, Lewis CM. Power of genetic association studies in the presence of linkage disequilibrium and allelic heterogeneity. *Hum Hered* 2008; **66**: 210-222
154 **Economou M**, Trikalinos TA, Loizou KT, Tsianos EV, Ioannidis JP. Differential effects of NOD2 variants on Crohn's disease risk and phenotype in diverse populations: a meta-analysis. *Am J Gastroenterol* 2004; **99**: 2393-2404
155 **Okazaki T**, Wang MH, Rawsthorne P, Sargent M, Datta LW, Shugart YY, Bernstein CN, Brant SR. Contributions of IBD5, IL23R, ATG16L1, and NOD2 to Crohn's disease risk in a population-based case-control study: evidence of gene-gene interactions. *Inflamm Bowel Dis* 2008; **14**: 1528-1541
156 **Brant SR**, Shugart YY. Inflammatory bowel disease gene hunting by linkage analysis: rationale, methodology, and present status of the field. *Inflamm Bowel Dis* 2004; **10**: 300-311

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