Genetic susceptibility for Inflammatory bowel diseases
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Chapter 1

Introduction and outline of the thesis
Introduction

Chronic inflammatory bowel diseases (IBD) comprising Crohn’s disease (CD) and ulcerative colitis (UC) are characterized by chronic relapsing inflammation of the gastrointestinal tract. The combined prevalence of CD and UC is estimated at 100 – 200 / 100,000 in developed countries. The pathogenesis of IBD is only partially understood but concordance rates in twins and siblings suggest that a genetic predisposition, apart from environmental and immunological factors, contributes to the pathogenesis of IBD. In the past decade, tremendous progress has been achieved in unraveling the genetic etiology of IBD. By conducting genome wide scans, several susceptibility loci for IBD have been identified. In 2001 the CARD15 gene encoding for the NOD2 protein on chromosome 16 (IBD1) has been found to be strongly associated with CD susceptibility. NOD2 is part of the innate immune system and is an intracellular pathogen-associated molecular pattern (PAMP) receptor that recognizes specific bacterial membrane components. Two missense mutations (R702W and G908R) and one frameshift insertion mutation (L1007fsinsC) in the leucine rich repeat region of the protein are independently associated with ileal CD in Caucasian patients. The L1007fsinsC mutation causes a truncated protein, suggesting that a defect in bacterial recognition might be involved in CD. The exact mechanism how mutations in CARD15 are involved in IBD susceptibility is still only partially understood and studies thus far give conflicting data. Although the discovery of CARD15 as a susceptibility gene has not led to clinical consequences, important progress and insight in the pathogenesis of IBD has been made. Actually, the discovery of the CARD15 gene in IBD has been one of the success stories in genetic research in complex genetic diseases and it has helped researchers to focus at signaling pathways of bacterial products in both epithelial and immune cells in the gut. Since CD and UC have many different phenotypic presentations and genome wide scans detected several linkage regions, IBD is considered a polygenic disease. Therefore many additional candidate-gene studies have been performed in the last decade.

**CODE Study**

For genetic research in complex genetic disorders as IBD it is important to have large homogenous cohorts of well described patients. In 2001 the CODE study (Chronische Ontsteking van de Darm en Erfelijkheid: Chronic Inflammation of the Gut and Inheritance) started in the University Medical Center Groningen. DNA has been, and is still being collected to form a large cohort of mainly Caucasian patients and family members from the Northern part of the Netherlands. Since the immigration rate in the Northern part of the Netherlands has been relatively low, this cohort can be considered a founder population. It is therefore suitable for genetic research and in particular for haplotype analysis, since it is assumed that the present population descends from a limited number of founders that results in evolutionary conserved haplotypes. Previously, several association studies have been performed in this cohort. For CARD15 the R702W and the 1007fsinsC mutation were independently associated with CD and not with UC. For different subsets of CD, association was found for an early age of onset, ileal localization, familial occurrence of IBD and penetrating or stricturing disease behavior. In another study in collaboration with the University Medical Center Nijmegen and
the Erasmus Medical Center Rotterdam association analysis was performed for IBD and Toll Like Receptor 4 (TLR4). TLR4 is, like NOD2, part of the innate immune system and is involved in NF-κB regulation. Haplotype analysis showed an association of CD and UC with TLR4, but in contrast with prior publications, no association could be found for the Asp266Gly and the Thr399Ile polymorphisms and IBD. Furthermore a candidate gene study was carried out for association of IBD and the Multi Drug Resistant 1 (MDR1) Gene, but no association could be found with IBD, CD, UC or different subsets of patients.

Genotypes vs. Phenotypes
An important aspect in studying IBD genetics is the consequent description of disease phenotypes. Since IBD is considered a multigenic disorder, different genes are probably involved in different subsets of phenotypes. It is therefore mandatory to have internationally accepted classification systems for IBD. An accepted and frequently used system is the Vienna classification. It includes age of onset, disease localisation and disease behaviour. A number of studies, including a study from the CODE cohort has validated this classification. However, several considerations have led to an update of the Vienna classification system during an expert meeting in Montreal in 2005. The main modifications were the introduction of an early age of onset category (< 16 years), the possibility of co-classification of upper gastrointestinal involvement and the inclusion of perianal disease as a disease modifier instead of being a form of penetrating disease. For the current thesis the original Vienna classification has been used.

Pharmacogenetics
Pharmacogenetics is another research subject in IBD genetics. There has been much interest in the pharmacogenetics of azathioprine metabolism. Azathioprine is a purine analogue that is frequently used in the treatment of Crohn’s disease but its use is hampered by the frequent occurrence of side-effects. Polymorphisms in the thiopurinemethyltransferase (TPMT) gene, which metabolizes azathioprine to 6-mercaptopurine and 6-methyl-mercaptopurine, and inosine triphosphate pyrophosphatase (ITPase) deficiency which leads to accumulation of the metabolite 6-thio-ITP, have been found to be responsible for a subset of the side-effects of azathioprine therapy.
Aims and outline of the thesis

This thesis aims to gain insight in the genetic background of IBD.

The first part of this thesis focuses on specific genetic associations with IBD. Therefore, a detailed review of the current literature on IBD-genetics is given in Chapter 2. Since the discovery of the association of CARD15 and IBD, many additional genes have been studied. Several of these genes are potentially truly associated, but results have been conflicting for many of the associations found. Next to specific genetic associations, current research on the functional role of mutations in CARD15 in IBD is also reviewed.

The initial part of the thesis comprises two studies investigating novel candidate genes for IBD susceptibility. In chapter 3 the association between IBD and Interleukin Receptor associated Kinase-M (IRAK-M) is studied. IRAK-M is a NF-κB-mediated, negative regulator of Toll-like receptor (TLR) signaling and is localized on chromosome 12q14, a susceptibility locus for IBD. It was hypothesized that a functional mutation in a negative regulator of TLR signaling might induce impaired endotoxin tolerance and increased inflammatory responses. Therefore IRAK-M is a good candidate gene for association analysis with IBD. 542 patients with IBD (309 CD and 233 UC) and 305 controls were studied. Phenotypic details of all CD patients according to the Vienna classification were available. UC patients were phenotyped according to an accepted classification including extend of the disease, age of onset, need for colectomy, extraintestinal manifestations and the occurrence of malignancy. Two single nucleotide polymorphisms (SNPs) and six microsatellite markers were evaluated by association analysis and Haplotype Sharing Statistics. Results were stratified for CARD15 mutations R702W, G908R and 1007fsinsC.

In Chapter 4 the genetic association between RUNX3 and IBD is studied. RUNX3 is a member of the runt domain family of transcription factors. It is known that loss of RUNX3 function is associated with a spontaneous colitis in knockout mice. It is a member of the TGF-β signaling pathway, which is a potent inhibitor of inflammation in IBD. Impaired activation of RUNX3 might result in decreased activity of the TGF-β pathway and decreased inhibition of inflammation in IBD. The gene encoding for RUNX3 resides on chromosome 1p36, which is a susceptibility locus for IBD. Therefore RUNX3 is a good candidate gene for susceptibility for IBD. Four SNPs and four microsatellite markers were studied for RUNX3 in the CODE cohort. Furthermore, mutations in SLC22A4 and 5 encoding for the organic cation transporters 1 and 2 (OCTN1/2) were found to be associated with CD in previous publications and an association was found between polymorphisms in SLC22A4, resulting in a disrupted binding site for RUNX in rheumatoid arthritis. For that reason, association analysis for 6 SNPs in SLC22A4/5 (including the known polymorphisms 207 G→C, 1672 C→G) and IBD and interaction with RUNX3 was studied. All results were stratified for CARD15 status. In addition to the genetic association analysis RUNX3 and OCTN1 expression was analyzed in colonic and ileal, inflamed and non-inflamed mucosal tissue samples of 30 IBD patients and 6 controls.
The second part of the thesis comprises three studies aimed at the confirmation of previously described genetic associations with IBD and describes specific genotype-phenotype interactions. Next to \textit{CARD15}, \textit{SLC22A4/5} and \textit{TLR4}, several other genes have been identified to be associated with IBD susceptibility. Simultaneously with the identification of \textit{SLC22A4/5}, genetic variations in \textit{DLG5} (Drosophila Discs Large Homologue 5) on chromosome 10q23 showed association with CD.\textsuperscript{7} DLG5 is important in maintaining epithelial stability and genetic variants could result in an impaired intestinal permeability. Additionally, two recent important studies identified two novel CD associated genes by performing the first genome wide association studies.\textsuperscript{8,9} An uncommon coding SNP in the gene encoding for the interleukin-23 receptor (\textit{IL23R}) conferred strong protection against CD. It was also shown to be associated with UC in non-Jewish patients. The other SNP in the autophagy-related 16-like 1 gene (\textit{ATG16L1}) was shown to be associated with CD.

It is supposed that genetic susceptibility has a more prominent role in the aetiology of early-, than of late-onset IBD, since early-onset patients were less exposed to environmental factors than late-onset patients. As a result, a higher frequency of IBD associated mutations is expected. In chapter 5 polymorphisms of \textit{CARD15}, \textit{TLR4}, \textit{SLC22A4/5} and \textit{DLG5} are analyzed in a cohort of 103 pediatric onset and 696 adult onset IBD patients and controls. Prevalence of mutations in the pediatric cohort was compared with the prevalence in adult-onset IBD and controls. Specific genotype-phenotype associations were studied.

Since it is of pivotal importance that genetic associations are confirmed in independent cohorts from different countries, chapter 6 describes a replication study for the two most strongly associated SNPs in \textit{IL23R} and \textit{ATG16L1} in our cohort of IBD patients. We were also interested in discovering whether these two genes are more generally involved in other common chronic disorders of the gastrointestinal tract and we therefore included a cohort of celiac disease patients from the Netherlands.

It is not only mandatory that previously found associations are confirmed in independent cohorts, these cohorts also need to have sufficient power to detect specific genotype-phenotype interactions. For \textit{SLC22A4/5} and \textit{DLG5} there have been conflicting results in the literature, but many studies are hampered by small sample size or the lack of adequate uniform phenotypic descriptions. As mentioned before, for genetic research in complex genetic disorders as IBD it is important to have large homogenous cohorts of well described and uniformly phenotyped patients. For that reason a large nationwide collaborative project was initiated. Results are described in chapter 7. DNA samples and phenotypic details of IBD patients from seven University Medical Centers in the Netherlands (University Medical Center Groningen; Academic Medical Center, Amsterdam; VU University Medical Center, Amsterdam; Leiden University Medical Center; Radboud University Nijmegen Medical Center; Erasmus Medical Center, Rotterdam and the University Medical Center Utrecht) were collected. 2937 patients (1696 CD, 1099 UC and 142 with indeterminate colitis) and 1484 healthy controls were included. Phenotypic details were available for 2090 patients (1315 CD / 775 UC). We performed an association analysis for \textit{DLG5}, \textit{SLC22A4/5} and \textit{ATG16L1} with IBD, CD, UC en different subsets of CD and UC. Interaction between these genes was studied.
The last part of the thesis focuses on azathioprine toxicity. In daily clinical practice it was noted that azathioprine toxicity occurred more often in IBD compared to other diseases for which azathioprine is frequently used. Particularly azathioprine induced pancreatitis had been rarely observed in other diseases than Crohn’s disease in clinical practice as well as in the literature. To investigate this clinical observation, a retrospective case-note review has been performed analyzing azathioprine toxicity and necessity of withdrawal in 1564 patients with a liver or renal transplantation, systemic lupus erythematosus, Wegener’s granulomatosis, autoimmune hepatitis, rheumatoid arthritis ulcerative colitis or Crohn’s disease. Azathioprine use and toxicity in the University Medical Center Groningen were also compared to the use in IBD patients in a large community hospital (Martini Hospital Groningen). Results are described in chapter 8. The fact that azathioprine induced acute pancreatitis was more prevalent in Crohn’s disease compared to other diseases, led to the exploration of an association of azathioprine induced pancreatitis and circulating pancreatic antibodies, which are highly specific for CD compared to UC and other autoimmune diseases.

Finally the results of the studies in this thesis are summarized and future perspectives for genetic research in IBD are given in chapter 9.
References


