Summary

Crohn's disease (CD) and ulcerative colitis (UC) are diseases collectively known as Inflammatory Bowel Disease (IBD). They are characterised by chronic relapsing inflammation of the digestive tract. Based on epidemiological data it is assumed that they are complex genetic disorders with multiple genes and environmental factors contributing to the pathogenesis. In 2001 an association between CD and the *Caspase-Activating Recruitment Domain containing protein 15* (*CARD15*) gene was reported. Individuals carrying the single nucleotide polymorphisms (SNPs) R702W, G908R and 1007fsinsC in *CARD15* showed to have a two to four-fold increased risk to develop CD. The publications that followed, assessing *CARD15* genotype-phenotype correlations in CD, showed several but inconsistent associations. The fact that many studies applied different definitions or used a limited number of variables may have contributed to these differences. The establishment of *CARD15* as a susceptibility gene for CD was a major step forward in IBD genetic research. Although the influence on daily practice is limited, as *CARD15* cannot be used as a diagnostic tool and has not yet resulted in novel therapeutic agents, it has led to the proposal of other candidate genes. One of these is the *Toll-like receptor 4* (*TLR4*) gene that was subject of study in this thesis. Linkage methods used to identify genes underlying rare Mendelian syndromes fail to find genes that contribute to more common, familial, non-Mendelian diseases. In the last decade new methods have been employed to find genes involved in complex genetic diseases. Instead of families and twins, founder populations can be studied. Individuals in founder populations share large fragments of DNA with each other that originate from common ancestors from centuries ago. Therefore, founder populations may be analysed as a large pedigree. The population in the northeastern part of the Netherlands can be considered to be a founder population. In founder populations, in addition to traditionally used SNPs, microsatellite markers can be employed for studying haplotypes. Microsatellite markers are more polymorphic than SNPs and provide therefore more information on the evolutionary history of the region covering the gene than SNPs. The Haplotype Sharing Statistic (HSS) is a method that analyses the differences in length of haplotype similarity between the patient and the control haplotypes. It is expected to be more powerful than single locus analysis, unless the studied SNPs would be causal themselves.

In genetic association studies of complex genetic diseases like IBD, detailed phenotypes need to be defined. Different genetic backgrounds may explain different clinical patterns in IBD. In recent years the Vienna classification, a simple list for defining objective parameters in patients with Crohn's disease that is widely accepted, was introduced in order to make studies on CD comparable.

The aim of this thesis was to study possible associations between certain candidate genes and UC, CD and subtypes of CD that are based on disease characteristics defined in detail.

Chapter 1 starts with an introduction of this thesis.
Chapter 2 reviews the genetics of IBD. Current evidence of genetic susceptibility in IBD and recent developments in this area are described. Emphasis has been put on the approaches and methods that are used to find genes that are associated with IBD.
In **chapter 3** a large cohort of CD patients, participating in the genetic association studies that are reported in the other chapters, is described. In order to be able to perform association analyses of CD subtypes, the clinical characteristics of CD patients within this population were determined in detail. To determine the usefulness of the Vienna classification in patient care and clinical studies, the outcome of disease was assessed in relation to clinical characteristics of patients with CD, as defined by the Vienna classification. Used outcome parameters, reflecting the severity of disease, were the rate and timing of operation, the occurrence of vitamin B12 deficiency and medication use. Also the side effects of immunosuppressive medication were assessed. A total of 292 patients with CD that were included were analysed. The age distribution of the incidence of CD showed a bimodal pattern. Delay of diagnosis until the time of the first operation occurred in one third of cases. Disease localisation in the ileocolon and anal involvement were associated with a more severe outcome as compared to localisation in the terminal ileum or the colon only. Also stricturing and penetrating disease behaviour was associated with a higher rate of operation whereas only patients with penetrating disease were administered infliximab more often. Need for surgery occurred early in the course of disease and if re-operations were needed they were performed within 5 years after the first operation in a majority of cases. Vitamin B12 deficiency was not only found in patients with disease involving the ileum, but also in patients with disease localisation in the colon only. In patients with ileal involvement, we did not detect a difference in vitamin B12 deficiency between patients that underwent ileocecal resection and those who were not operated. Immunosuppressive agents were prescribed in more than half of the patients during the course of disease, mostly azathioprine. Intolerance of azathioprine, including the occurrence of pancreatitis, was observed frequently.
In **chapter 4** the association between *CARD15* and IBD is described. In order to confirm the association between CD and R702W, G908R and 1007fsinsC in our population, the association between these SNPs and IBD was tested. To determine the association between these variants and subtypes of CD, an association analysis was performed between these variants and phenotypic features in thoroughly phenotyped CD patients. To identify possible other mutations in or nearby *CARD15* that are associated with CD or UC, we also tested six microsatellite markers that were analysed in combination with the SNPs. In addition, in order to elucidate the inconsistent findings of genotype-phenotype studies a pooled analysis of *CARD15* IBD association studies was performed. In our IBD population we found an association of CD with R702W and 1007fsinsC. Genotype-phenotype analysis showed association with several characteristics. Among these characteristics were age of onset less than forty years, ileal localisation, complicated disease characteristics and familial occurrence. In an additional association analysis with six microsatellite markers we found a strong association between marker NOD-04 and

CD. This marker was in linkage disequilibrium with the polymorphisms R702W, G908R and 1007fsinsC. The haplotype analysis also showed strong association with CD. No association was found with UC. In the pooled analysis all three common *CARD15* variants showed strong association with CD and not with UC. Phenotype analysis showed association with small bowel involvement, stricturing and penetrating disease.
The results of the association analysis of IBD with *TLR4* are described in **chapter 5**. *TLR4* is involved in the innate immune system and like *CARD15* it plays a role in NF-κB regulation. A functional variant of *TLR4* has been described to affect the response to lipopolysaccharides and *TLR4* is strongly upregulated in epithelial cells in IBD patients. We therefore hypothesised that *TLR4* could be involved in IBD. Association analysis between variants of the two polymorphisms Asp299Gly and Thr399Ile and IBD and particular CD phenotypes was performed. In addition four microsatellite markers flanking *TLR4* were studied. We found neither association between the Asp299Gly and Thr399Ile variants and IBD, nor between these variants and CD and UC. However, we did find an association between these variants and a subgroup of CD cases with a disease onset at the age of forty years or older. No interaction with *CARD15* variants was observed. Haplotype analysis, performed with the Haplotype Sharing Statistic (HSS), of both SNPs and the microsatellite markers, showed association between marker D9S1864 and both IBD and CD and between marker TLR406 and UC. It is hypothesised that Asp299Gly and Thr399Ile are in linkage with the disease susceptibility mutation located elsewhere on *TLR4*.
In **chapter 6** the results of the association study of the *Multidrug Resistance (MDR)1* gene are given. *MDR1* is found to be associated with colitis in animal studies and in humans a reduced *MDR1* mRNA expression is found in UC patients. We hypothesised that *MDR1* is an IBD susceptibility gene. We tested the association between *MDR1* and IBD. Six SNPs and six microsatellite markers, all in and close to *MDR1*, were studied for association with IBD and its subgroups UC and CD and particular CD subgroups. Using single locus association analysis of the SNPs and haplotype analyses, including the HSS, no association could be observed for any of the groups. There was also no interaction with *CARD15* variants. This indicates that there is no association between *MDR1* and IBD.