GENETIC VARIATION AND CLINICAL MANIFESTATIONS IN INFLAMMATORY BOWEL DISEASE

Maarit Lappalainen

Department of Medicine and
Research Program for Molecular Medicine,
Biomedicum Helsinki,
University of Helsinki, Finland

ACADEMIC DISSERTATION

To be publicly discussed with the permission of the Faculty of Medicine, University of Helsinki, in Lecture Hall 2, Haartman Institute, Haartmaninkatu 3, on 23rd May 2008, at 12 o'clock.

Helsinki 2008

Supervised by

Paulina Paavola-Sakki, MD, PhD
Department of Medicine
University of Helsinki
Helsinki, Finland

and

professor Kimmo Kontula, MD, PhD
Department of Medicine
University of Helsinki
Helsinki, Finland

Reviewed by

docent Irma Järvelä, MD, PhD
Department of Medical Genetics
University of Helsinki
Helsinki, Finland

and

docent Kaija-Leena Kolho, MD, PhD
Hospital of Children and Adolescents
University of Helsinki
Helsinki, Finland

Official opponent

docent Jukka Partanen, PhD
Research and Development
Finnish Red Cross Blood Service
Helsinki, Finland

ISBN 978-952-92-3448-6 (paperback)
ISBN 978-952-10-4566-0 (pdf)
http://ethesis.helsinki.fi

Helsinki University Printing House
Helsinki 2008

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications:

I Heliö T, Halme L, **Lappalainen M**, Fodstad H, Paavola-Sakki P, Turunen U, Färkkilä M, Kontula K. *CARD15/NOD2* Gene Variants Are Associated with Familially Occurring and Complicated Forms of Crohn's Disease. *Gut* 2003; 52(4):558-62.

II **Lappalainen M**, Paavola-Sakki P, Halme L, Turunen U, Färkkilä M, Repo H, Kontula K. Novel *CARD15/NOD2* Mutations in Finnish Patients with Crohn's Disease, and Their Relation to Phenotypic Variation *in vitro* and *in vivo*. *Inflammatory Bowel Diseases* 2008; 14(2):176-85.

III Hugot JP**,** Zaccaria I, Cavanaugh J**,** Yang H**,** Vermeire S, **Lappalainen M**, Schreiber S, Annese V, Jewell DP, Fowler EV, Brant SR, Silverberg MS, Cho J, Rioux JD, Satsangi J, Parkes M, for the IBD International Genetics Consortium. Prevalence of *CARD15/NOD2* Mutations in Caucasian Healthy People. *American Journal of Gastroenterology* 2007; 102(6):1259-67.

IV **Lappalainen M**, Paavola-Sakki P, Halme L, Turunen U, Heliö T, Färkkilä M, Kontula K. Screening of Tumor Necrosis Factor Receptor-Associated Factor 6 (TRAF6) as a Candidate Gene for Inflammatory Bowel Disease. *Scandinavian Journal of Gastroenterology* 2006; 41(4):424-9.

V **Lappalainen M**, Halme L, Turunen U, Saavalainen P, Einarsdottir E, Färkkilä M, Kontula K, and Paavola-Sakki P. Association of *IL23R*, *TNFRSF1A*, and HLA-DRB1*0103 Allele Variants with Inflammatory Bowel Disease Phenotypes in the Finnish Population. *Inflammatory Bowel Diseases* 2008; Mar 13 [epub ahead of print].

The publications are referred to in the text by their Roman numerals I-V and are reproduced with the permission of the copyright holders.

ABBREVIATIONS

ABCB1	ATP-binding cassette, subfamily B, member 1
ASCA	anti-*Saccharomyces cerevisiae* antibody
ATG16L1	autophagy 16-like 1
bp	base pair
CARD15	caspase recruitment domain-containing protein 15
CD	Crohn's disease
DLG5	discs large, Drosophila, homolog of, 5
CRP	C-reactive protein
ELISA	enzyme-linked immunosorbent assay
ESR	erythrocyte sedimentation rate
GI	gastrointestinal
GWA	genome-wide association
dHPLC	denaturing high-performance liquid chromatography
HEK	human embryonic kidney
HLA	human leukocyte antigen
IBD	inflammatory bowel disease
IL23R	interleukin 23 receptor
kb	kilobase
LD	linkage disequilibrium
LRR	leucine-rich repeat
Mb	megabase
MDP	muramyl dipeptide
MDR1	multidrug resistance 1
MHC	major histocompatibility complex
NBD	nucleotide binding domain
NOD2	nucleotide-binding oligomerization domain protein 2
NLR	NOD-like receptor
NPL	nonparametric linkage
OCTN1	organic cation transporter 1
OCTN2	organic cation transporter 2
pANCA	perinuclear anti-neutrophil cytoplasmic antibody
PAMP	pathogen-associated molecular pattern
PGN	peptidoglycan
PIRA	primer-induced restriction assay
PRR	pattern recognition receptor
RFLP	restriction fragment length polymorphism
SLC22A4	solute carrier family 22, member 4
SLC22A5	solute carrier family 22, member 5
SNP	single nucleotide polymorphism
TLR4	toll-like receptor 4
TNFRSF1A	tumor necrosis factor receptor superfamily, member 1a
TNFSF15	tumor necrosis factor superfamily, member 15
TRAF6	TNF receptor-associated factor 6
UC	ulcerative colitis
UTR	untranslated region

In addition, standard one-letter abbreviations are used for nucleotides and amino acids.

ABSTRACT

Crohn's disease (CD) and ulcerative colitis (UC), two major forms of inflammatory bowel disease (IBD), are chronic relapsing conditions of the gastrointestinal tract. IBD prevalence in Finland is approximately 3-4 per 1000 and the peak age of onset is between 20 and 35 years of age. The precise aetiology of IBD is unknown but interplay of environmental risk factors and immunologic changes trigger the disease in a genetically susceptible individual. Twin and family studies have provided strong evidence for genetic factors in IBD susceptibility, and genetic factors may be more prominent in CD than UC. The first CD susceptibility gene was identified in 2001. Three common mutations R702W, G908R, and 1007*fs* of the *CARD15/NOD2* gene are shown to associate independently with CD but the magnitude of association varies between different populations.

One of the main objectives of this study was to evaluate the role of *CARD15* in a Finnish CD cohort. 271 CD patients were studied for the three common mutations and the results showed a lower mutation frequency than in other Caucasian populations. Only 16% of the patients carried one of the three mutations. Ileal location as well as stricturing and penetrating behaviour of the disease were associated with occurrence of the mutations. The whole protein coding region of *CARD15* was screened for possible Finnish founder mutations. In addition to several sequence variants, five novel mutations (R38M, W355X, P727L, W907R, and R1019X) were identified in five patients. Functional consequences of these novel variants were studied *in vitro*, and these studies demonstrated a profound impairment of MDP response. Investigation of *CARD15* mutation frequency in healthy people across three continents showed a large geographic fluctuation. No simple correlation between mutation frequency and disease incidence was seen in populations studied. The occurrence of double mutant carriers in healthy controls suggested that the penetrance of risk alleles is low.

Other main objectives aimed at identifying other genetic variations that are involved in the susceptibility to IBD. We investigated the most plausible IBD candidate genes including *TRAF6*, *SLC22A4*, *SLC22A5*, *DLG5*, *TLR4*, *TNFRSF1A*, *ABCB1/MDR1*, *IL23R*, and *ATG16L1*. The marker for a chromosome 5 risk haplotype and the rare HLA-DRB1*0103 allele were also studied. The study cohort consisted of 699 IBD patients (240 CD and 459 UC), of which 23% had a first-degree relative with IBD. Of the several candidate genes studied, *IL23R* was associated with CD susceptibility, and *TNFRSF1A* as well as the HLA-DRB1*0103 allele with UC susceptibility. *IL23R* variants also showed association with the stricturing phenotype and longer disease duration in CD patients. In addition, *TNFRSF1A* variants were more common among familial UC and ileocolonic CD.

In conclusion, the common *CARD15* mutations were shown to account for 16% of CD cases in Finland. Novel *CARD15* variants identified in the present study are most likely disease-causing mutations, as judged by the results of *in vitro* studies. The present study also confirms the *IL23R* association with CD susceptibility and, in addition, *TNFRSF1A* and HLA-DRB1*0103 allele association with UC of specific clinical phenotypes.

INTRODUCTION

Inflammatory bowel disease or IBD usually refers to either Crohn's disease (CD) or ulcerative colitis (UC). Case descriptions of diseases that today would probably be called IBD were published as far back as several centuries ago (Russel 2000). In 1913, doctor Thomas Kennedy Dalziel reported thirteen patients suffering from intestinal obstruction in the *British Medical Journal* (Hawkins 1990). On autopsy he found that all patients had an inflamed gut and the inflammation was transmural, which is today known as one of the characteristics of CD. Between the 1920s and 1930s more young people with CD like symptoms were seen. In 1932, doctors Crohn, Ginsberg, and Oppenheimer published their findings in *The Journal of the American Medical Association*, and mainly because of the timing CD came to be known as a medical entity.

In 1859 Sir Samuel Wilks described the first case of UC in a letter to the *Medical Times and Gazette* even though at that time he did not know the correct diagnosis (Hawkins 1990). Later, in 1875, Wilks with Walter Moxon described UC showing that the disease had a non-infectious cause. Already at the beginning it was thought that CD and UC are two distinct conditions. At present, in about 10% of cases the distinction between these two disorders can not be made and the term indeterminate colitis is used.

Before the human genome was sequenced mainly the genes responsible for rare Mendelian disorders were successfully found. It has been more difficult to find disease-causing gene variants for complex diseases like IBD because the effect of individual genes is small and gene interactions play an essential role. In addition, environmental factors are important in disease development. In genetics, recent advances in genotyping technologies and determination of the human genome sequence have made a huge impact on resolving the genetic causes of common disorders. Establishment of repositories of large patient samples is also a powerful tool in common disease genetics. Twin and family studies have shown that genetic susceptibility is essential in IBD development, nevertheless only a few IBD susceptibility genes have been identified to date.

In the present study, genetics of two inflammatory bowel diseases, Crohn's disease and ulcerative colitis, and possible correlation of genetic variations to clinical characteristics were investigated in an IBD cohort of Finnish origin.

REVIEW OF THE LITERATURE

1. INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) is a group of inflammatory conditions affecting the gastrointestinal (GI) tract. The two main forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC). Typically, the onset of IBD is in late adolescence or early adulthood (Russel and Stockbrugger 1996; Farrokhyar *et al.* 2001) with equal ratios of men and women in most reports. The symptoms of IBD include diarrhoea, abdominal pain, fever, and weight loss. However, CD and UC can usually be distinguished by clinical and pathologic characteristics. The main difference between these two disorders is the location and nature of the inflammation (Table 1) (Bouma and Strober 2003). In CD, the inflammation can involve any part of the GI tract from mouth to anus, but it most commonly affects the small intestine (jejunum and/or ileum) and colon. UC instead affects only the colonic part of the GI tract, sometimes only the rectum. Microscopically, inflammation in CD is transmural affecting the whole bowel wall, while in UC it is restricted to the mucosal layer.

Table 1. Differentiation between Crohn's disease and ulcerative colitis (Lennard-Jones 1989; Baumgart and Sandborn 2007).

Clinical features	Crohn's disease	Ulcerative colitis
Colon involvement	Usually	Always
Terminal ileum involvement	Commonly	Seldom
Rectum involvement	Seldom	Usually
Involvement around anus	Common	Seldom
Distribution of inflammation	Patchy	Continuous
Nature of inflammation	Transmural	Superficial
Ulcers in endoscopy	Serpiginous and Deep	Superficial
Fistulas	Common	Seldom
Stenosis	Common	Seldom
Crypt abscesses on biopsy	Uncommon	Common/Always
Granulomas on biopsy	Common	Uncommon

Both diseases may have similar extraintestinal manifestations usually affecting liver (primary sclerosing cholangitis, autoimmune hepatitis), skin (erythema nodosum, pyoderma gangrenosum), eyes (uveitis, iritis), and joints (arthritis, ankylosing spondylitis) (Rothfuss *et al.* 2006). In about 10% of IBD cases it is not possible to distinguish between CD and UC, and then the term indeterminate colitis is used.

1.1 Crohn's disease

Crohn's disease (CD, also known as regional enteritis, granulomatous ileitis, and ileocolitis) is named after American gastroenterologist Burrill B. Crohn. In CD, the main symptoms include abdominal pain, diarrhoea, bloody stools, pain in defecation, rectal bleeding and pain, fever, and weight loss (Farmer *et al.* 1975; Hanauer and Sandborn 2001; Carter *et al.* 2004). Many patients have symptoms for years before a diagnosis is made. The symptoms also vary between patients depending on the part of the gut affected. Phenotypic classification of the disease is based on the location and behaviour of the inflammation. Vienna classification is a simple classification of CD based on a set of objective variables including age of onset (Table 2) (Gasche *et al.* 2000). In almost half of the cases, CD affects both the ileum and the colon (ileocolitis) (Farmer *et al.* 1985). In around thirty percent of cases only the small bowel (jejunoileitis or ileitis) is affected and in the remaining twenty percent the large intestine (colitis) is affected. The upper GI tract, esophagus, stomach, or duodenum is affected in about ten percent of cases with or without the inflammation in the small intestine or colon. Behaviour of this condition can be divided into three categories: stricturing, penetrating, and inflammatory. Stricturing behaviour (stenosis) causes narrowing of the bowel that may lead to bowel obstruction. The penetrating form of the disease creates fistulas between the bowel and other organs such as the skin. Fistulas occur most commonly around the anal area. Patients with a pure inflammatory form of CD do not develop either fistulas or strictures. The anatomical location of CD is fairly stable whereas the behaviour varies substantially during the natural disease course (Baumgart and Sandborn 2007).

Table 2. Vienna classification of patients with Crohn's disease.

Variable	
Age at onset	1. < 40 years 2. ≥ 40 years
Disease location	1. terminal ileum 2. colon 3. ileocolon 4. upper gastrointestinal tract
Disease behaviour	1. inflammatory 2. stricturing 3. penetrating

CD activity indexes have been developed to standardise and quantify disease severity. Crohn's Disease Activity Index (CDAI) from 1976 uses eight different clinical variables in the determination of disease activity (Best *et al.* 1976). Index values below 150 indicate remission and values above 450 are an indication of severe disease. A few years later the simplified CDAI was developed (Harvey and Bradshaw 1980). This simplified index,

Harvey–Bradshaw Index (HBI, Simple Index) is based on only five variables. Both the Vienna classification and the CDAI/HBI are mainly used in scientific research for the determination of disease severity. Most recently, fecal calprotectin and lactoferrin have been shown to be more sensitive surrogate markers than normally used activity indexes (Sipponen *et al.* 2008). Regarding disease activity, only 10-13% of patients will remain in remission for several years (Munkholm *et al.* 1995; Loftus *et al.* 2002). About 70% of patients have a chronic intermittent course while in the remainder the disease is constantly active.

1.2 Ulcerative colitis

In ulcerative colitis (UC) the colon is affected by the formation of ulcers. The patients usually present with diarrhoea mixed with blood and mucus and sometimes also abdominal pain, urgency, or tenesmus (Carter *et al.* 2004; Kornbluth and Sachar 2004; Baumgart and Sandborn 2007). As in CD, the symptoms may occur on and off for a long period of time. UC is also classified according to the extent of the inflammation. This depends on how far up the colon the disease extends. The inflammation usually begins from the rectum. In simple classification the disease is divided into a distal or extensive disease (Kornbluth and Sachar 2004). Distal colitis can be further divided into proctitis (only the rectum affected), proctosigmoiditis (the rectosigmoid colon affected), or left-sided colitis (the descending colon affected) (Baumgart and Sandborn 2007). In pancolitis, the inflammation covers the whole colon extending from the rectum to the cecum, and sometimes even furher to the distal ileum – a phenomenon termed as backwash ileitis.

In addition to the extent of the involvement, UC can be categorised by the severity of the disease. Disease severity is defined as mild, moderate, severe, or fulminant (Truelove and Witts 1955; Hanauer 1996; Carter *et al.* 2004; Silverberg *et al.* 2005). In mild cases, there are less than four stools per day, and in moderate disease stool frequency is more than four with minimal signs of toxicity. Severe UC is manifested by more than six bloody stools daily and toxicity is demonstrated by fever, tachycardia, anaemia, or an elevated erythrocyte sedimentation rate (ESR). Patients with fulminant disease usually have more than ten bowel movements per day with continuous bleeding, toxicity, abdominal tenderness and distension, and require blood transfusions and colonic dilation. During the natural course of the disease it may progress proximally (Langholz *et al.* 1994; Henriksen *et al.* 2006). Disease activity in the overall patient population is remarkably constant with about half of the patients in clinical remission at any given time, but the majority of patients have an intermittent course (Langholz *et al.* 1994). In addition, approximately

20% and 40% of UC patients did not have a relapse or were not taking medication, respectively, five years after diagnosis (Henriksen *et al.* 2006).

1.3 Diagnosis

There is no single test for IBD. Diagnosis is a combination of clinical symptoms, radiologic or endoscopic appearance of the bowel together with typical histology and exclusion of other causes of intestinal inflammation (Lennard-Jones 1989; Hanauer and Sandborn 2001; Baumgart and Sandborn 2007). Usually the diagnosis between CD and UC is clear but in approximately 10% of cases the distinction can not be made and the term indeterminate colitis is used. With time the symptoms or clinical findings become clearer which makes the differential diagnosis between these two conditions possible. Colonoscopy is a primary tool for diagnostics since it allows direct visualization from the rectum to the terminal ileum. A wireless capsule endoscopy can be used in CD diagnosis to evaluate the extent of the small bowel involvement. In this procedure, a small capsule with a built-in camera is swallowed and the camera takes serial pictures of the entire small bowel, finally ending up in the patient's faeces. In CD, the inflammation is discontinuous whereas in UC it is continuous. In addition, deep ulcers may be present in CD.

During the endoscopy, several biopsies are taken from various parts of the bowel in order to confirm the diagnosis and to assess the inflammation activity. CD shows a transmural pattern of inflammation and granulomas are present in about 50% of CD cases. In UC histopathology, the inflammation is more diffuse and restricted to mucosal layers. The pathology typically involves distortion of crypt architecture and inflammation of crypts (cryptitis).

C-reactive protein (CRP) correlates well with deep inflammation in the bowel wall in CD (Vermeire *et al.* 2006). Bleeding and malabsorption cause anaemia, which may be due to iron, folate, or vitamin B_{12} deficiencies. Laboratory tests in mild and moderate UC cases are usually normal (Silverberg *et al.* 2005). Stool samples for calprotectin and lactoferrin measurements have been used in IBD diagnosis for a few years now (Sipponen *et al.* 2008). However, sometimes in moderate and always in the most severe cases blood tests show elevated acute-phase reactants CRP and ESR, leukocytosis, thrombocytosis, and hypoalbuminemia. Stool cultures need to be performed in both diseases to rule out parasites and infectious causes.

Although there are no specific diagnostic laboratory tests for IBD, UC and CD can be characterised by different serological antibody expression. High levels of perinuclear

antineutrophil cytoplasmic antibodies (pANCA) in the serum are strongly correlated with occurrence of UC (Shanahan *et al.* 1992; Seibold *et al.* 1994; Lee *et al.* 1995; Halme *et al.* 2002) while serum reactivity to anti-*Saccharomyces cerevisiae* antibodies (ASCA) is associated with CD (Lee *et al.* 1995; Quinton *et al.* 1998; Sutton *et al.* 2000; Seibold *et al.* 2001; Halme *et al.* 2002). However, these tests do not reliably separate the two diseases. They have uncertain value in indeterminate colitis cases and are not recommended for routine diagnosis.

1.4 Treatment

The treatment of IBD is sequential: first, the treatment of acute disease in order to achieve remission and then the maintenance of remission. In addition, minimising the side effects of the treatment and improving quality of life are other equally important factors. Remission is usually achieved by medication. Medical treatment is dependent on the disease course, extent of inflammation, and disease severity. Patients with IBD often require a combination of drugs to control their disease (Irving *et al.* 2008). However, in the mildest cases patients may not need any treatment. In both disorders, medical therapy consists of anti-inflammatory drugs such as 5-aminosalicylic acid (5-ASA) and corticosteroids as well as immunosuppressive agents (azathioprine or its derivatives, methotrexate, cyclosporine), sometimes also antibiotics. 5-ASA can be used in the treatment of mild IBD as well as in the induction and maintenance of remission (Carter *et al.* 2004; Kornbluth and Sachar 2004). Corticosteroids are effective in moderate to severe UC and in reducing the symptoms of CD. Because of side-effects, the prolonged use of corticosteroids should be avoided. Drug treatment in severe IBD consists of corticosteroids, which should be reduced over time and then later switch on to immunosuppressants, if needed. Although some patients are able to maintain remission with 5-ASA alone, many require immunosuppressive drugs (Hanauer and Sandborn 2001). The precise role of antibiotics in the treatment of IBD is unknown, however, in clinical practise, antibiotics are frequently used (Perencevich and Burakoff 2006). Antibiotics may be useful in the treatment of CD patients with colonic disease (Sutherland *et al.* 1991). The proven involvement of cytokines in the IBD pathophysiology has led to the development of anticytokine drugs, so-called biologics, as a therapy for IBD over the last decades. Among biological therapies, anti-TNF antibodies (infliximab or adalimumab) play a pivotal role in the treatment of chronic active IBD and fistulating disease when other medical treatments fail to induce remission (Kuhbacher and Folsch 2007).

Unresponsiveness to medical treatment and complications are the main reasons for surgery. Around 55-90% of CD patients ultimately require an operation (Farmer *et al.*

1976; Hanauer and Sandborn 2001; Larson and Pemberton 2004). Indications for surgery are usually intestinal obstruction, internal fistula and abscess, or poor response to medical therapy (Farmer *et al.* 1976). Perforation of the bowel and severe bleeding may sometimes necessitate surgery. Resections in CD should be, however, conservative (Carter *et al.* 2004). Resection of the involved bowel may improve the symptoms but does not cure the disease. Unlike CD, UC can be cured by surgically removing the large intestine and rectum (proctocolectomy). Most patients with UC will not require surgery but approximately one third will eventually need an operation (Larson and Pemberton 2004). Emergency colectomy is indicated in case of massive hemorrhage, severe toxic megacolon as a result of fulminanat colitis, or perforation (Berg *et al.* 2002; Cima and Pemberton 2005). Another indication for surgery is unresponsiveness to medication (Larson and Pemberton 2004; Cima and Pemberton 2005). In both disorders, documented or strongly suspected dysplasia and carcinoma are indications for elective surgery.

1.5 Epidemiology

The incidence of IBD has increased dramatically in the western world since the early 1950s (Russel 2000; Jess *et al.* 2007; Loftus *et al.* 2007), with a peak onset between 20 and 35 years of age. The highest incidence rates have been reported for North America, the United Kingdom, and Scandinavia, whereas lower rates are found in southern Europe, Africa, Asia, and South America (Farrokhyar *et al.* 2001; Loftus 2004; Lakatos 2006; Baumgart and Carding 2007). The overall annual incidence rate for CD varies between 0.1-16/100 000 inhabitants and for UC between 0.5-24.5/100 000 inhabitants (Ouyang *et al.* 2005; Jacobsen *et al.* 2006; Lakatos 2006; Lapidus 2006). The corresponding IBD prevalence reaches as high as 396/100 000 inhabitants (Ouyang *et al.* 2005; Lakatos 2006). CD and UC prevalence varies between 26-213/100 000 and 37-294/100 000 inhabitants, respectively (Loftus 2004; Jacobsen *et al.* 2006; Lapidus 2006).

Geography has a strong influence on IBD incidence, with higher incidence in the northern than southern hemisphere (Garland *et al.* 1981; Sonnenberg *et al.* 1991; Sonnenberg and Wasserman 1991; Shivananda *et al.* 1996; Loftus *et al.* 1998; Bernstein *et al.* 1999; Loftus *et al.* 2000; Russel 2000). IBD is also more common in developed and industrialized countries as well as in urban areas (Russel and Stockbrugger 1996; Loftus *et al.* 2000; Russel 2000; Lakatos 2006; Baumgart and Carding 2007). It has recently been observed that the gap between areas of high and low incidences is diminishing, possibly as a result of a changing, more westernised, life-style (Russel 2000). It is also of note that CD incidence is still increasing in many areas whereas UC incidence has been stable for some time (Farrokhyar *et al.* 2001) and in some areas CD has become more prevalent than UC

(Molinie *et al.* 2004). Despite the stabilisation of incidence, the prevalence still continue to grow as a result of the early age of onset and low mortality (Loftus *et al.* 2007).

In a European collaborative study during 1991-93, both UC and CD incidences were higher in the northern than southern Europe (Shivananda *et al.* 1996). According to this study the overall incidence in Europe was 10.4/100 000 individuals for UC and 5.6/100 000 for CD. UC rates in northern centres were 40% higher than those in the south and for CD they were up to 80% higher, but this gradient was narrower than previously believed. In addition, it seems that continuously increasing incidences are still reported in high incidence areas such as Denmark (Vind *et al.* 2006), Sweden (Lapidus 2006), and Finland (Turunen *et al.* 2006). In Finland, the IBD prevalence was 240/100 000 inhabitants in 1993 (Karvonen *et al.* 1993). In 2006, however, according to drug compensation statistics from the Social Insurance Institution of Finland (KELA), the IBD prevalence had inreased to over 500/100 000. Among children, the IBD incidence has almost doubled in the last 20 years (Turunen *et al.* 2006), so it is obvious that the trend is similar among adults, although there are no reports on that. The annual CD incidence during 1996-2000 was 5.6/100 000 and the corresponding prevalence was 79.7/100 000 (Jussila *et al.* 2004). The latest UC incidence and prevalence rates are from 1993 when the corresponding rates were 8.1/100 000 and 147/100 000, respectively (Karvonen *et al.* 1993).

2. GENETIC EPIDEMIOLOGY

Epidemiological, clinical, and molecular studies have provided strong evidence that inherited predisposition is important in the pathogenesis of chronic IBD. IBD is a complex disorder with an aetiology that is only partly understood. In complex disorders, both genetic and environmental factors have a role in the development of the disease. Unlike Mendelian inheritance, in complex diseases genes are expected to have a low penetrance. Therefore, genes are less prone to selection which results in higher gene frequencies than expected. IBD is now widely believed to originate from a dysregulated immune response to luminal bacteria in a genetically susceptible individual (Abreu and Sparrow 2006; Bamias and Cominelli 2007; Van Limbergen *et al.* 2007b). The genetic predisposition in IBD has long been known since epidemiological studies have shown that the prevalence of IBD is increased among relatives with CD and UC.

2.1 Twin studies

Twin studies have been a powerful tool for the identification of different contributions of genes and environmental factors in the aetiology of complex diseases. If a disease was to

be entirely due to genes, like in monogenic diseases, the concordance would be 100% in identical (monozygotic) twins and 50% in non-identical (dizygotic) twins. In contrast, if the disease was entirely dependent on non-inherited factors, then the concordance would be similar in both twin pairs. Strong evidence for the involvement of genetic factors in IBD derives from twin studies which have shown significantly higher concordance rates in monozygotic than in dizygotic twins. Large twin studies from Sweden, Denmark, and the United Kingdom show that the concordance rate for CD was 20-58% in identical twins and less than 10% in non-identical twins when brought up in same environment (Tysk *et al.* 1988; Thompson *et al.* 1996; Orholm *et al.* 2000; Halfvarson *et al.* 2003; Russell and Satsangi 2004). The corresponding rates for UC were 6-19% in monozygotic twins and 4% in dizygotic twins (Halfvarson *et al.* 2003; Russell and Satsangi 2004). These numbers show that the genetic influence is stronger in CD than in UC.

2.2 Family studies

Since the first observation of familial occurrence in 1963, family studies have shown significant familial clustering of IBD (Kirsner and Spencer 1963; Farmer *et al.* 1980; Orholm *et al.* 1991; Satsangi *et al.* 1994; Peeters *et al.* 1996). A positive family history is the most important independent risk factor for the disease. In addition, the greatest risk is to develop the same type of IBD as the affected relative. Figures vary from one study to another, but approximately 10-15% of adult IBD patients have a family member affected with IBD (Bonen and Cho 2003). The risk is particularly strong among first-degree relatives. CD patients have a first-degree relative with CD in 2.2-16.2% of cases and with IBD in 5.2-22.5% of cases (Russell and Satsangi 2004). In UC patients, the corresponding figures are 5.7-15.5% with UC and 6.6-15.8% with IBD. At highest risk are children of two affected parents (>30%) (Laharie *et al.* 2001). A positive family history is more common in CD than in UC (Farmer *et al.* 1980; Satsangi *et al.* 1994). Earlier disease onset in the offspring of IBD patients is consistently found, and genetic anticipation has been hypothesized (Polito *et al.* 1996b). This phenomenon, however, may be a result of a combination of a time trend, increasing incidence of IBD. Mixed families also exist where both CD and UC occurs within the same family, at a frequency greater than expected by chance alone, indicating that these two are closely related disorders and may even share some susceptibility genes.

A degree of familial clustering of a disorder may be expressed as a ratio (λ_s) of a risk for siblings to the reported population prevalence. Using this λ_s value as a relative risk, the risk range is 15-42 for CD and 7-17 for UC (Satsangi *et al.* 1997; Ma *et al.* 1999; Ahmad *et al.* 2001). These λ_s values for CD are even higher than for many other complex

diseases, including type 1 and 2 diabetes (15 and <10), celiac disease (7-30), and schizophrenia (8.6) (Todd and Farrall 1996; Ahmad *et al.* 2001; Pena and Wijmenga 2001; Halme *et al.* 2006). This information shows that genes are a strong contributor to the development of CD in particular.

2.3 Racial and ethnic groups

Considerable differences exist in the incidence and prevalence of IBD between different racial and ethnic groups (Roth *et al.* 1989; Yang *et al.* 1993). The highest prevalence is seen among Caucasians, lower prevalence rates in black Americans, and the lowest rates in Hispanics and Asians (Satsangi *et al.* 1994; Duerr 2003; Baumgart and Carding 2007). Among Caucasians the highest incidence and prevalence rates are observed in Jewish populations with two to four times higher than in non-Jewish Whites (Yang *et al.* 1993; Polito *et al.* 1996a; Vermeire and Rutgeerts 2005). Within Jewish populations, rates of IBD are greater in Ashkenazi Jews than in Sephardic/Oriental Jews (Gilat *et al.* 1974; Rozen *et al.* 1979; Krawiec *et al.* 1984; Odes *et al.* 1987a; Odes *et al.* 1987b). And even among Ashkenazi Jews the rates for IBD are greater in Jews of central European origin than in those of Polish and Russian origin.

2.4 Interaction between genes and environment

IBD is currently believed to result from the complex effect of diverse genes conferring a risk of disease and environmental factors, which when combined lead to an aberrant inflammation response in the gut (Ahmad *et al.* 2001; Ferguson *et al.* 2007; Yamamoto-Furusho and Podolsky 2007). The discovery of the *CARD15* gene in the CD pathogenesis (Hampe *et al.* 2001; Hugot *et al.* 2001; Ogura *et al.* 2001a) has refocused attention on innate immunity and the interaction between genetic factors and bacterial flora. The innate immune response is the first line of defence against invading pathogens (Aderem and Ulevitch 2000; Schwartz 2002; Bourhis *et al.* 2007) and the human intestinal tract is exposed to an enormous microbial flora all the time. The mucosal immune system has evolved to balance the need to respond to pathogens while co-existing with commensal bacteria and food antigens (Yamamoto-Furusho and Podolsky 2007). Innate immune responses are initiated by activation of pattern recognition receptors (PRRs), which consist of NOD-like receptor (NLR) and toll-like receptor (TLR) protein family members (Philpott and Girardin 2004; Bourhis *et al.* 2007; Kanneganti *et al.* 2007; Franchi *et al.* 2008). Immediate activation of innate immunity relies on detection of conserved microbial motifs known as pathogen associated molecular patterns (PAMPs), comprising diverse molecules from bacteria and viruses such as lipopolysaccharide (LPS), peptidoglycan

(PGN), flagellin, lipoprotein, and viral double-stranded RNA (Barton and Medzhitov 2003; Akira *et al.* 2006).

Of exogenous factors, smoking is the most consistent finding in IBD showing that smokers are at a higher risk of developing CD than non-smokers (Tobin *et al.* 1987; Lindberg *et al.* 1988; Logan and Kay 1989; Silverstein *et al.* 1989; Corrao *et al.* 1998; Halme *et al.* 2002; Garcia Rodriguez *et al.* 2005; Halfvarson *et al.* 2006). On the other hand, smoking has been shown to have a protective effect against UC (Benoni and Nilsson 1987; Tobin *et al.* 1987; Logan and Kay 1989; Rutgeerts *et al.* 1994; Parrello *et al.* 1997; Corrao *et al.* 1998; Halme *et al.* 2002; Garcia Rodriguez *et al.* 2005; Firouzi *et al.* 2006; Halfvarson *et al.* 2006; de Saussure *et al.* 2007). Some studies have also suggested appendectomy, use of oral contraceptives, breastfeeding, high sugar and fat diet, infections in childhood and vaccination as risk factors for IBD but the results are controversial (Farrokhyar *et al.* 2001; Loftus 2004).

3. MOLECULAR GENETIC STUDIES

3.1 Candidate gene approach

In the early years attempts to identify IBD susceptibility genes focused primarily on candidate gene studies. Today this approach is still used although more sophisticated methods have emerged. The candidate gene approach can be used when there is some pre-existing knowledge about the trait studied (Tabor *et al.* 2002). There are two main types of candidate genes, functional and positional. Functional candidates are genes with a putative role in disease development. Studies with animal models, association with other closely related diseases, information about pathways, and gene expression data can all increase understanding of the potential function of candidate genes (Cho and Weaver 2007). In contrast, positional candidate genes are identified through genome-wide scans. In this approach, genetic variations within the gene of interest are typed with polymorphic markers or haplotype tagging SNPs in the study population and the frequency of the variation is compared between patients and controls. Another possibility is to resequence the gene of interest in the study population in order to detect all variations within the gene. Variations can be divided into coding or non-coding SNPs, amino acid substitutions, small insertions or deletions, and copy number variations (CNVs). These variations can have an effect on the particular gene by altering the structure or expression of the encoded protein.

The most studied functional candidate genes in IBD susceptibility have been the human leukocyte antigen (HLA) molecules, based on their role in regulating the immune response

and well-established association with other autoimmune diseases such as celiac disease, rheumatoid arthritis, and diabetes. *TLR4* and tumor necrosis factor-α (TNF-α) as functional IBD candidate genes as well as genes of interleukin-1 (IL-1) family have also been investigated in the pathogenesis of IBD based on their role in immune response (Orchard *et al.* 2000). The most remarkable example of the positional candidate gene approach has been the identification of the *CARD15* gene in CD susceptibility (Hampe *et al.* 2001; Ogura *et al.* 2001a).

3.2 Genome-wide linkage studies

A genome-wide linkage approach can be applied in families with multiple affected individuals when there is no previous information about the susceptibility genes. In the past, linkage studies have been successfully used to identify the genes underlying rare monogenic Mendelian disorders (Baron 2001; Hirschhorn and Daly 2005). The principle behind a genome-wide scan is to genotype highly informative markers, usually microsatellite markers, distributed over the whole genome and follow whether a certain marker is inherited together with a disease phenotype within a family. If the causal gene is located close enough to the marker it is likely to be co-inherited at meiosis. Markers that are closest to the disease gene show the strongest correlation, indicated as LOD (logarithm of odds) or NPL (non-parametric linkage) scores, with the disease phenotype in families. Genome-wide linkage analysis became feasible due to development of microsatellite marker maps and semi-automated genotyping techniques over the last decade. Between the years 1996-2004 all together 11 IBD genome-wide linkage scans were performed (Hugot *et al.* 1996; Satsangi *et al.* 1996a; Cho *et al.* 1998; Hampe *et al.* 1999a; Ma *et al.* 1999; Duerr *et al.* 2000; Rioux *et al.* 2000; Williams *et al.* 2002; Paavola-Sakki *et al.* 2003; Barmada *et al.* 2004; Vermeire *et al.* 2004). In addition, there is a meta-analysis using ten of the published genome-wide scans (van Heel *et al.* 2004).

To date there are nine IBD loci identified through linkage studies with varying degrees of replication and statistical support (Table 3). While some loci seem to be specific to CD or UC, others seem to confer susceptibility to IBD overall. The most consistent linkage finding is linkage between CD and the *IBD1* locus in the pericentromeric region of chromosome 16, discovered in the first genome scan in 1996 (Hugot *et al.* 1996). The *IBD1* locus in CD susceptibility has been replicated in many subsequent linkage studies (Ohmen *et al.* 1996; Brant *et al.* 1998; Cavanaugh *et al.* 1998; Curran *et al.* 1998; Annese *et al.* 1999; Hampe *et al.* 1999a; Cavanaugh 2001) with the greatest replication of linkage (MLOD 5.79) with 613 nuclear families provided by the IBD International Genetics Consortium (Cavanaugh 2001). It is presently known that the *CARD15* gene in this region

confers susceptibility to CD, but *CARD15* does not explain all the linkage seen at this locus. Linkages to the *IBD3* and *IBD5* loci are the next most consistent linkage findings in IBD genetics, and their role is described in more detail in section 5.

Table 3. IBD susceptibility loci identified by genome-wide linkage studies.

LOCUS	CHROMOSOME	DISEASE	GENES	REFERENCES
IBD1	16q12	CD	*CARD15*	Hugot *et al.* 1996, Hampe *et al.* 1999, Williams *et al.* 2002, Paavola-Sakki *et al*. 2003
IBD2	12p13.2-q24.1	UC		Satsangi *et al.* 1996a, Hampe *et al.* 1999, Ma *et al.* 1999, Paavola-Sakki *et al.* 2003, Barmada *et al.* 2004
IBD3	6p	IBD	HLA class II	Hampe *et al.* 1999, Rioux *et al.* 2000, Williams *et al.* 2002, Barmada *et al.* 2004, van Heel *et al.* 2004
IBD4	14q11-q12	CD		Ma *et al.* 1999, Duerr *et al.* 2000, Vermeire *et al.* 2004
IBD5	5q31	CD	*SLC22A4/5*	Ma *et al.* 1999, Rioux *et al.* 2000, Williams *et al.* 2002
IBD6	19p13	IBD		Rioux *et al.* 2000, Paavola-Sakki *et al.* 2003
IBD7	1p36	IBD		Cho *et al.* 1998
IBD8	16p	CD		Hampe *et al.* 2002a*
IBD9	3p26	IBD		Satsangi *et al.* 1996a, Rioux *et al.* 2000

*Not a genome-wide linkage study

The first evidence for significant linkage in UC was detected in the second genome-wide scan (Satsangi *et al.* 1996a). This locus, named *IBD2*, was located on chromosome 12 with a significant LOD score of 5.47. This finding has later been replicated in many genome-wide scans (Hampe *et al.* 1999a; Ma *et al.* 1999; Paavola-Sakki *et al.* 2003; Barmada *et al.* 2004). Studies on the *IBD2* locus have also shown linkage to both CD and UC (Duerr *et al.* 1998; Yang *et al.* 1999a; Parkes *et al.* 2000; Uthoff *et al.* 2002; Achkar *et al.* 2006). Despite several studies of candidate genes including interferon gamma (*IFNG*) (Hampe *et al.* 1998), vitamin D receptor (*VDR*) (Simmons *et al.* 2000; Martin *et al.* 2002), integrin beta-7 (*ITGB7*) (van Heel *et al.* 2001), and signal transducer and activator of transcription 6 (*STAT6*) (Xia *et al.* 2003), no susceptibility gene responsible for the *IBD2* linkage has yet been identified.

3.2.1 Genome-wide linkage study in Finnish IBD families

A genome-wide scan in Finnish families with IBD provided evidence for novel susceptibility loci (Paavola-Sakki *et al.* 2003). This study was unique since the UC cohort

examined was the largest analysed from one population so far. Furthermore, it provided data on the genetically homogenous Finnish isolate which may have advantages in the mapping of complex disease genes. In the first phase of the study, 92 IBD families were genotyped for 429 microsatellite markers spaced at approximately 10 cM intervals. The second phase consisted of high density mapping of chromosomal regions 2p13-11, 11p12-q13, and 12p13-12 with 38 new families and 15 additional markers. The most significant two-point NPL scores for UC were 2.61 on chromosome 2p11 and 2.00 on 12p13. The highest two-point NPL score of 2.15 for IBD was also reached on the same 12p13 locus and the second best value of 2.0 was obtained on chromosome 11. For CD, the highest two-point NPL score was 2.34 on chromosome 12q23. In addition, a nominal multipoint NPL score of 2.11 was observed for chromosome 16. When *CARD15* positive families were excluded, a multipoint NPL score of 1.57 was still observed at the same location. When the IBD cohort was stratified according to early age at onset, the linkage signals were further improved on chromosomes 2p11, 11p12-q13, 12p13, 19p13, and 19q13. The corresponding multipoint NPL scores were 2.34, 2.31, 2.19, 2.07, and 2.66, respectively. This study replicated the linkages at the *IBD1*, *IBD2*, and *IBD6* loci (Table 3) and provided some evidence for novel loci on chromosomes 2, 11p12-q13, 12q23, and 19q13.

3.3 Genome-wide association studies

Genome-wide association (GWA) studies are becoming an increasingly popular tool for identifying complex disease genes with low or modest effects in large sample sets. The popularity of this method is due to advances in technology for high-throughput genetic analysis, with very low error rates and low genotyping costs (Amos 2007). Progress made by the Human Genome Project and the International HapMap Project have also helped to make GWA studies possible (Hirschhorn and Daly 2005; Cho and Weaver 2007). The study population in a GWA study consists of affected individuals and healthy controls which is generally much easier to obtain than a collection of families for linkage studies. The markers used in GWA studies are SNP markers and tests of association with the condition are conducted by comparing allele frequencies between patients and controls. All published GWA studies in IBD have so far focused on CD which is thought to be less genetically heterogeneous than UC (Yamazaki *et al.* 2005; Duerr *et al.* 2006; Consortium 2007; Hampe *et al.* 2007; Libioulle *et al.* 2007; Raelson *et al.* 2007; Rioux *et al.* 2007). Within a short period of time many novel candidate genes have been reported, at the moment the most replicated genes being *IL23R* and *ATG16L1* (Table 4).

Table 4. Novel candidate genes and chromosomal loci identified by genome-wide association studies.

GENE	dbSNP ID	CHROMOSOME	REFERENCES
TNFSF15	several SNPs	9q33	Yamazaki *et al.* 2005
IL23R	rs11209026	1p32.1-p31.2	Duerr *et al.* 2006, Libioulle *et al.* 2007, Consortium 2007, Raelson *et al.* 2007
ATG16L1	rs2241880	2q37.1	Hampe *et al.* 2007, Libioulle *et al.* 2007, Rioux *et al.* 2007, Consortium 2007
PTGER4	several SNPs	5p13.1	Libioulle *et al.* 2007, Consortium 2007
	rs224136	10q21.1	Rioux *et al.* 2007, Consortium 2007
PHOX2B	rs16853571	4p12	Rioux *et al.* 2007
NCF4	rs4821544	22q13.1	Rioux *et al.* 2007
FAM92B[*]	rs8050910	16q24.1	Rioux *et al.* 2007
IRGM	rs1000113	5q33.1	Consortium 2007
BSN, *MST1*	rs9858542	3p21	Consortium 2007, Raelson *et al.* 2007
NKX2-3	rs10883365	10q24.2	Consortium 2007
PTPN2	rs2542151	18p11	Consortium 2007
JAKMIP1, *LOC285484*	several SNPs	4p16.1	Raelson *et al.* 2007
	rs4794986	17q11.1	Raelson *et al.* 2007
	rs8077981	17q23.2	Raelson *et al.* 2007

[*]a predicted gene

4. IBD SUSCEPTIBILITY GENES

Over the years, several genes have been proposed in IBD susceptibility but they have demonstrated varying levels of replication by independent studies. Before GWA studies, *CARD15*, *IBD5* (includes *SLC22A4/5*), and HLA class II associations represented the most consistent replications (Table 5). For many of the candidate genes, either inadequate or conflicting replication studies have been reported and therefore definite conclusions regarding IBD association can not be made. *DLG5*, *ABCB1/MDR1*, and *TLR4* genes belong to this category of controversial results (Table 5). Two other candidate genes *TNFRSF1A* and *TRAF6* were of special interest due to functional properties of the genes and previous results from the genome-wide linkage study of Finnish IBD families (Table 5). Based on the recent GWA studies, the most promising susceptibility genes are *IL23R* and *ATG16L1* (Tables 4 and 5).

Even though there is still much to be discovered in the pathogenesis of IBD, genetic defects at different levels are suspected to lead to the development of chronic inflammation in IBD (Goyette *et al.* 2007). Mutations in environmental sensors, such as *CARD15* and *TLR4*, and their signalling pathways including *TRAF6* may alter the innate

Table 5. IBD susceptibility and most promising candidate genes. Only positive associations are shown here.

GENE	dbSNP ID	CHROMOSOME	DISEASE	POPULATION	REFERENCES
CARD15	rs2066844 rs2066845 rs5743293	16q12	CD	Belgian, British, Danish, Dutch, French, German, Hungarian, Irish, Israeli Arab, Italian, Jews, North American, Quebec, Scottish, Spanish, Swedish	Hampe *et al.* 2001, Hugot *et al.* 2001, Ogura et al. 2001a, Abreu *et al.* 2002, Ahmad *et al.* 2002, Cuthbert *et al.* 2002, Hampe *et al.* 2002b, Vermeire *et al.* 2002a, Vermeire *et al.* 2002b, Zhou *et al.* 2002, Bairead *et al.* 2003, Bonen *et al.* 2003, Sun *et al.* 2003, Arnott *et al.* 2004, Fernandez *et al.* 2004, Giachino *et al.* 2004, Heresbach *et al.* 2004, Karban *et al.* 2004, Lakatos *et al.* 2004, Newman *et al.* 2004, Tukel *et al.* 2004, Annese *et al.* 2005a, Jess *et al.* 2005, Lakatos *et al.* 2005, Vind *et al.* 2005, Bene *et al.* 2006, Ferraris *et al.* 2006, Oostenbrug *et al.* 2006b, Torkvist *et al.* 2006, Cucchiara *et al.* 2007b, Ernst *et al.* 2007, Riis *et al.* 2007
SLC22A4	rs1050152	5q31	CD	British, Greek, German, Italian, New Zealand, North American, Scottish, Spanish, Swedish	Peltekova *et al.* 2004, Gazouli *et al.* 2005a, Noble *et al.* 2005a, Torok *et al.* 2005, Babusukumar *et al.* 2006, Fisher *et al.* 2006, Leung *et al.* 2006, Martinez *et al.* 2006, Onnie *et al.* 2006a, Waller *et al.* 2006, Cucchiara *et al.* 2007b, Silverberg *et al.* 2007, Torkvist *et al.* 2007
SLC22A5	rs2631367	5q31	CD	Same as in *SLC22A4*	Same as in *SLC22A4* without study of Silverberg *et al.* 2007
DLG5	DLG5_e26rs	10q23	IBD	British,	Stoll *et al.* 2004, Daly *et*

	1248696			Canadian, German, Italian, New Zealand, Scottish	*al.* 2005, Newman *et al.* 2006, Browning *et al.* 2007, Russell *et al.* 2007
ABCB1/ MDR1	rs2032582 rs1045642 rs3789243	7q21.1	IBD	British, German, Iranian, North American, Scottish, Slovenian, Spanish	Brant *et al.* 2003, Potocnik *et al.* 2004, Ho *et al.* 2005, Annese *et al.* 2006, Ho *et al.* 2006, Onnie *et al.* 2006b, Urcelay *et al.* 2006, Farnood *et al.* 2007
TLR4	rs4986790 rs4986791	9q32-33	IBD	Belgian, Dutch, German, Greek,	Franchimont *et al.* 2004, Torok *et al.* 2004, Braat *et al.* 2005, Brand *et al.* 2005, Gazouli *et al.* 2005b, Ouburg *et al.* 2005, de Jager *et al.* 2007
TNFRSF1A	rs767455	12p13.2	IBD	Belgian, Canadian	Pierik *et al*. 2004, Waschke *et al.* 2005
TRAF6		11p12			
IL23R	rs1004819 rs7517847 rs10489629 rs2201841 rs11465804 rs11209026 rs1343151 rs10889677 rs11209032 rs1495965	1p32.1-31.2	IBD	Dutch, English, German, Hungarian, Italian, New Zealand, North American, Scottish, Spanish,	Baldassano *et al.* 2007b, Borgiani *et al.* 2007, Buning *et al.* 2007, Cummings *et al.* 2007a, Dubinsky *et al.* 2007, Glas *et al.* 2007, Oliver *et al.* 2007, Roberts *et al.* 2007, Tremelling *et al.* 2007, van Limbergen *et al.* 2007, Weersma *et al.* 2008
ATG16L1	rs2241880	2q37.1	CD	British, Dutch, German, New Zealand, North American, Scottish	Baldassano *et al.* 2007a, Cummings *et al.* 2007b, Prescott *et al.* 2007, Roberts *et al.* 2007, Glas *et al.* 2008, van Limbergen *et al.* 2008, Weersma *et al.* 2008

immunity by causing a decrease in NF-κB activation and defensin production. *DLG5* and *ABCB1/MDR1* gene mutations may change the permeability of the epithelial barrier. In addition, mutations in the genes involved in adaptive immunity including HLA genes and *TNFRSF1A* as well as cytokine receptor *IL23R* may cause an imbalance between

regulatory and effector cell immune responses which are important in controlling inflammatory reactions.

4.1 *CARD15*: the first CD susceptibility gene

An initial link between chromosome 16 and CD was reported in 1996 (the *IBD1* locus, Table 3) (Hugot *et al.* 1996). Five years later, the IBD International Genetics Consortium replicated the linkage on chromosome 16 (Cavanaugh 2001). Twelve groups from Europe, North America, and Australia provided 613 nuclear families which showed strong linkage to multiple markers on chromosome 16 with a peak LOD score of 4.96. When analysis was restricted to CD families only, a LOD score of 5.79 was detected in the same interval with no evidence of linkage in UC and mixed families. These results indicated that the gene(s) at the *IBD1* locus likely conferred susceptibility solely for CD.

Later the same year, three independent groups reported that mutations in the caspase recruitment domain-containing protein 15 (*CARD15*) gene are associated with CD (Hampe *et al.* 2001; Hugot *et al.* 2001; Ogura *et al.* 2001a). This was a landmark discovery since *CARD15* was the first susceptibility gene known to be involved in a complex disease and it also confirmed the previous epidemiological data suggesting that CD is a genetic disorder. The first two studies were reported independently in the same issue of *Nature* (Hugot *et al.* 2001; Ogura *et al.* 2001a) and the third study a month later in *The Lancet* (Hampe et al. 2001). Hugot and co-workers proceeded from the linkage observation to *CARD15/NOD2* gene identification by positional-cloning strategy whereas the two other groups adopted *CARD15* as a potential candidate gene. Three common variants, R702W, G908R, and 1007*fs*, were shown to associate with CD susceptibility, the 1007*fs* mutation being the most significant (Hugot *et al.* 2001). Analysing the results further it was observed that these three mutations were always on different haplotypes. The relative risk of developing CD in heterozygotes was estimated to be three-fold, and in homozygotes or compound heterozygotes about 40 fold. Ogura and co-workers, who had just identified the *CARD15* gene (Ogura *et al.* 2001b), came to the same conclusion showing that the R702W, G908R, and 1007*fs* mutations are responsible for CD susceptibility (Ogura *et al.* 2001a). In the third study, the 1007*fs* mutation was shown to associate with CD in German and British cohorts (Hampe *et al.* 2001).

4.1.1 *CARD15* structure, expression, and function

CARD15 encodes an intracellular nucleotide-binding oligomerization domain protein 2 (NOD2) receptor involved in regulating proinflammatory cytokines in response to bacteria

by inducing signalling pathways of nuclear factor-κB (NF-κB) and mitogen-activated protein kinases (MAPKs) (Akira and Takeda 2004; Strober *et al.* 2006; Cho and Abraham 2007; Kanneganti *et al.* 2007). NOD2 belongs to the NLR family that contains an N-terminal caspase recruitment domain (CARD), a central nucleotide binding domain (NBD also known as NACHT), and a C-terminal regulatory leucine-rich repeat (LRR) domain (Ogura *et al.* 2001b; Martinon and Tschopp 2005; Creagh and O'Neill 2006). The CARD domain is responsible for protein-protein interactions generating downstream signalling, the NBD domain mediates self-oligomerization and also has ATPase activity, and the LRR domain is involved in ligand recognition directly or indirectly (Strober *et al.* 2006; Bourhis *et al.* 2007). NOD2, being a pattern recognition receptor (PRR) in innate immunity, recognises muramyl dipeptide (MDP) in the cytoplasm (Bonen *et al.* 2003; Chamaillard *et al.* 2003; Girardin *et al.* 2003; Inohara *et al.* 2003). MDP is derived from the bacterial cell wall component peptidoglycan (PGN) which is a common component of both gram-positive and gram-negative bacteria.

When *CARD15* was found, it was at first thought that its expression was restricted to peripheral blood monocytes (Ogura *et al.* 2001b). Later it was shown that NOD2 is also expressed in various other tissues such as dendritic cells (Gutierrez *et al.* 2002), endothelial cells (Davey *et al.* 2006), granulocytes (Gutierrez *et al.* 2002), intestinal myofibroblasts (Otte *et al.* 2003), Paneth cells of the small intestine (Lala *et al.* 2003; Ogura *et al.* 2003), and various epithelial cells including intestinal epithelium (Berrebi *et al.* 2003; Hisamatsu *et al.* 2003; Uehara *et al.* 2007).

4.1.2 NOD2 signalling pathway

Mechanisms by which muramyl dipeptide (MDP) enters the cytoplasm appear to be via a transporter protein hPepT1 (Vavricka *et al.* 2004; Ismair *et al.* 2006), by infection of invasive bacteria, through phagocytosis or secretion system-mediated translocation of muropeptides, and by host-cell mediated internalisation (Philpott and Viala 2004; Strober *et al.* 2006; Bourhis *et al.* 2007). Inside the cell MDP is recognised by the LRR domain of NOD2 (Figure 1). NOD2 has been shown to localise to cytoplasm, vesicles, and plasma membrane (Barnich *et al.* 2005; Lecine *et al.* 2007). The membrane association of NOD2 makes it possible to respond to MDP and activate the NF-κB pathway. NOD2 is also in charge of the membrane recruitment of a serine-threonine kinase RICK (also called RIP2, RIPK2 and CARDIAK), the next molecule in the pathway. RICK, in turn, binds directly to IκB kinase (IKK) γ (also known as NEMO) and activates the IKK complex by promoting the ubiquitinylation of IKKγ and by stimulating the kinase activity of IKKα and

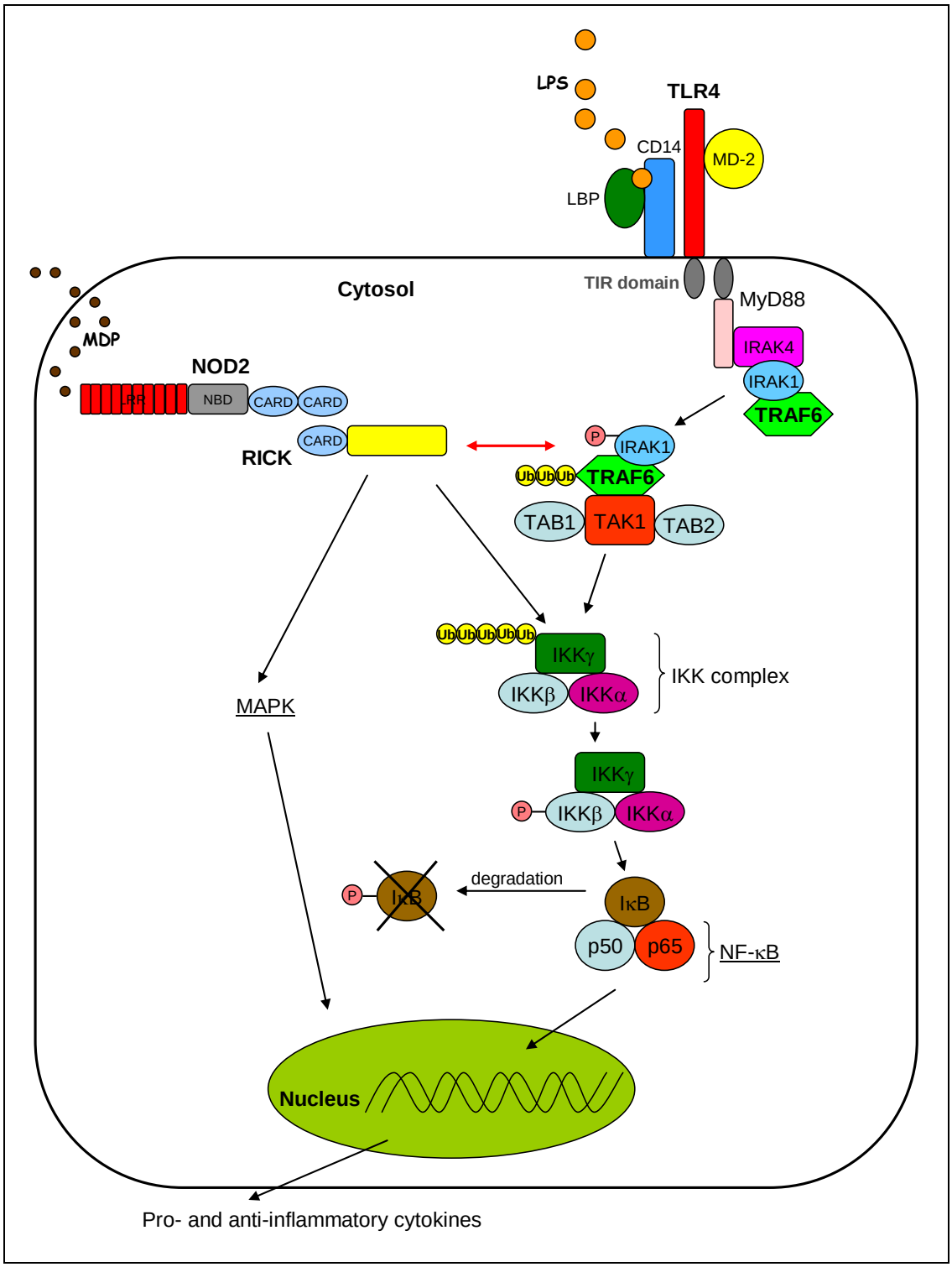

Figure 1. NOD2 and TLR4 signalling pathways in antigen presenting cell (APC) resulting in production of pro- and anti-inflammatory cytokines. Possible interactions between these two pathways are indicated with a red arrow. NOD2 downstream signalling is mediated through a CARD domain and TLR4 signalling through a cytosolic TIR domain (Toll/interleukin-1 receptor). CD14=monocyte differentiation antigen CD14, IKK=I-kappa-B kinase, IRAK=interleukin 1 receptor-associated kinase, LBP=lipopolysaccharide-binding protein, LPS=lipopolysaccharide, MAPK=mitogen-activated protein kinase, MDP=muramyl dipeptide, MyD88=myeloid differentiation primary response gene 88, NOD2=nucleotide-binding oligomerization domain protein 2, NF-κB=nuclear factor-κB, P=phosphorylation, RICK=RIP-like interacting clarp kinase, TAB=TAK-binding protein, TAK1=transforming growth factor-beta-activated kinase 1, TLR4=toll-like receptor 4, TRAF6=TNF receptor-associated factor 6, Ub=ubiquitination.

IKKβ, the other two components of the IKK complex (Inohara *et al.* 2000; Abbott *et al.* 2004). Next, the phosphorylation of IKKβ and IκB leads to the release of NF-κB for translocation to the nucleus and production of proinflammatory cytokines. In addition to NF-κB, pathogen-induced expression and secretion of multiple cytokines is mediated through several transcription factors activated by MAPK-related kinases (Inohara *et al.* 2005). NOD2 induces activation of p38, JNK, and ERK but the exact molecular mechanism by which the activation is achieved is not well understood at the moment.

The NOD2 dependent signalling pathway is far from being fully characterised. NOD2 acts independently of the TLR cascade but it is likely that interplay between these two protein families ensures the efficient co-ordination of innate immune responses, through either synergistic or co-operative signalling (Figure 1) (Netea *et al.* 2004; Watanabe *et al.* 2004; Meinzer and Hugot 2005; van Heel *et al.* 2005b; Bourhis *et al.* 2007). One proposed model of interaction between these pathways is at the level of TAK1 as RICK has been shown to induce the activation of TAK1 (Windheim *et al.* 2007). The most recent study suggests that the signalling pathways converge at the level of TRAF6, which is known to be activated by NOD2 (Abbott *et al.* 2007).

4.1.3 *CARD15* mutation frequency and genotype-phenotype associations in different CD cohorts

Investigations of the three common mutations R702W, G908R, and 1007*fs* in the *CARD15* gene have demonstrated a notable amount of heterogeneity across different populations. Ethnically divergent populations may exhibit the same phenotype but this may not be due to the same set of variants, genes, or even loci. *CARD15* variants are found in the majority of Caucasian CD patients (Table 5) although the mutation frequency in different populations is remarkably different. The overall frequencies of the common *CARD15* mutations in North American and central European Caucasian CD populations vary between 27.0% and 46.1% (Hugot *et al.* 2001; Abreu *et al.* 2002; Ahmad *et al.* 2002; Cuthbert *et al.* 2002; Hampe *et al.* 2002b; Vermeire *et al.* 2002a; Vermeire *et al.* 2002b; Bairead *et al.* 2003; Fernandez *et al.* 2004; Giachino *et al.* 2004; Heresbach *et al.* 2004; Lakatos *et al.* 2004; Newman *et al.* 2004; Annese *et al.* 2005a; Lakatos *et al.* 2005; Bene *et al.* 2006; Ferraris *et al.* 2006; Oostenbrug *et al.* 2006b; Riis *et al.* 2007). In Scotland, which is a high incidence population, the allele frequencies were lower still (22.8%) as compared to other central European populations (Arnott *et al.* 2004). The mutation frequency in children with CD seems to be even higher, ranging from 38% to 67% (Sun *et al.* 2003; Bene *et al.* 2006; Cucchiara *et al.* 2007b). This is an indication for stronger genetic predisposition in early-onset CD.

The relative risk for CD when carrying one common *CARD15* mutation is only about 2-4 fold whereas carriage of two mutations (compound heterozygote or homozygote) increases the relative risk dramatically from 15 to 40 fold (Hugot *et al.* 2001; Cuthbert *et al.* 2002; Economou *et al.* 2004; Newman *et al.* 2004; Vermeire and Rutgeerts 2005; Oostenbrug *et al.* 2006b; Henckaerts and Vermeire 2007).

Several studies have investigated the phenotypic expression of *CARD15* variants, and the most consistently replicated association is of *CARD15* mutations with ileal CD (Hampe *et al.* 2001; Abreu *et al.* 2002; Ahmad *et al.* 2002; Cuthbert *et al.* 2002; Hampe *et al.* 2002b; Lesage *et al.* 2002; Vermeire *et al.* 2002b; Bairead *et al.* 2003; Brant *et al.* 2003b; Arnott *et al.* 2004; Economou *et al.* 2004; Fernandez *et al.* 2004; Giachino *et al.* 2004; Heresbach *et al.* 2004; Lakatos *et al.* 2004; Annese *et al.* 2005a; Lakatos *et al.* 2005; Ferraris *et al.* 2006; Oostenbrug *et al.* 2006b). The stricturing and/or penetrating phenotype has also been associated with *CARD15* mutations (Abreu *et al.* 2002; Hampe *et al.* 2002b; Lesage *et al.* 2002; Brant *et al.* 2003b; Economou *et al.* 2004; Giachino *et al.* 2004; Heresbach *et al.* 2004; Lakatos *et al.* 2005; Oostenbrug *et al.* 2006b; van der Linde *et al.* 2007). In some studies, carriage of two mutations has been shown to associate with an earlier age at onset (Lesage *et al.* 2002; Brant *et al.* 2003b; Newman *et al.* 2004; Annese *et al.* 2005a; Oostenbrug *et al.* 2006b). Conflicting results concerning the allele frequencies between familial and sporadic cases have been reported. In some studies the frequency has not been different (Newman *et al.* 2004; Annese *et al.* 2005a; van der Linde *et al.* 2007) whereas other studies have been able to show higher allele frequencies in familial patients (Cuthbert *et al.* 2002; Zhou *et al.* 2002; Economou *et al.* 2004; Oostenbrug *et al.* 2006b).

In Scandinavia, CD incidence is among the highest in the world. However, the allele frequencies of the three common *CARD15* mutations have been reported to be lower than in central Europe and North America. Nevertheless, these variants have still been associated with CD as well as with specific phenotypes, namely earlier age at diagnosis (Ernst *et al.* 2007) and ileal disease location (Vind *et al.* 2005; Medici *et al.* 2006; Torkvist *et al.* 2006; Ernst *et al.* 2007). In Sweden, the common variants R702W and G908R, but not the 1007*fs* mutation, were associated with CD susceptibility (Torkvist *et al.* 2006). The contribution of the three mutations in Swedish CD patients was relatively low, with 15.2% of patients carrying one of the common mutations. *CARD15* mutation frequency in Swedish pediatric patients with CD was even lower, only 8.6% (Idestrom *et al.* 2005). This may be partly due to a lower frequency of ileal disease in this cohort. Study of Norwegian population for the three mutations showed a low mutation frequency of 7.0% (Hampe *et al.* 2002b). In a second Norwegian study, the mutation frequency was similar with no significant CD association (Medici *et al.* 2006). Indeed, the R702W and

G908R variants had essentially identical allele frequencies among Norwegian controls and cases. The third 1007*fs* had higher frequency in patients than in healthy controls (5.3% vs. 2.4%) but the difference was not statistically significant. In Denmark, approximately 21% of the CD patients studied carried at least one *CARD15* mutation (Vind *et al.* 2005; Ernst *et al.* 2007). The R702W and 1007*fs* mutations were also independently associated with CD in Danish patients. A recent publication reported a much higher *CARD15* mutation frequency of 44% in Danish twins with CD (Jess *et al.* 2005). There is a single study concerning the *CARD15* mutation frequency in Icelandic CD patients, where no one of 49 CD patients had any of the three common mutations (Thjodleifsson *et al.* 2003).

Jews have the highest incidence and prevalence rates of IBD compared to other ethnic groups. The mutation frequency of the *CARD15* gene has been investigated in different subgroups of Jews, namely Ashkenazi and Sephardic populations. In North American Ashkenazi Jewish populations, 30-34% of CD patients carried at least one of the three common mutations (Zhou *et al.* 2002; Bonen *et al.* 2003; Newman *et al.* 2004; Tukel *et al.* 2004). In one study, patients carrying two mutations were shown to have an earlier age at onset, especially for familial patients (Zhou *et al.* 2002). *CARD15* mutation frequency in Ashkenazi and Sephardic Jews from Israel has also been studied (Karban *et al.* 2004). The overall mutation frequency in CD patients was 41%, which is comparable to other Caucasian populations. A higher mutation frequency was observed in Ashkenazi compared to Sephardic Jews (48% vs. 28%). In another study, similar mutation frequency of 35% for a Sephardic Jewish population has been observed (Tukel *et al.* 2004).

The common *CARD15* mutations are not a major contributor to CD in Asian populations. Two papers from Japan were published soon after the *CARD15* finding and none of the main mutations was found in patient or control groups (Inoue *et al.* 2002; Yamazaki *et al.* 2002). Other negative reports on Asian populations involved Chinese (Leong *et al.* 2003; Gao *et al.* 2005) and South Korean (Croucher *et al.* 2003b; Lee *et al.* 2005) cohorts. These findings indicate that the three common *CARD15* mutations are quite new and have arisen after the Asian-European population split. A significantly lower *CARD15* mutation frequency has been identified in African-American compared to Caucasian cohorts (Bonen *et al.* 2002). Little is also known about African and South American populations with one exception of low mutation frequency in coloured South Africans (Zaahl *et al.* 2005).

4.1.4 Rare *CARD15* variants and their effect on phenotype

On average, 60-70% of CD patients do not carry any of the three *CARD15* mutations (Abreu *et al.* 2002; Ahmad *et al.* 2002; Cuthbert *et al.* 2002; Hampe *et al.* 2002a;

Vermeire *et al.* 2002a). These mutations do not account for all the linkage observed between CD and the *IBD1* locus (Hugot *et al.* 2001; Hampe *et al.* 2002a; Paavola-Sakki *et al.* 2003). Since the residual linkage at the *IBD1* locus was detected after removing the families with three variants, a search for additional disease-causing mutations in the coding region of the *CARD15* gene has also been conducted. The first comprehensive study comprised 453 CD patients (Lesage *et al.* 2002). The authors identified 67 sequence variants of which 27 were rare and considered as potential disease-causing mutations (Table 6). Three common mutations accounted for 81% of the CD associated *CARD15* variants, whereas the remaining 19% consisted of rare mutations. In addition, 50% of CD patients carried one mutation and 17% had two mutations, confirming the gene-dosage effect. Patients with two mutations more frequently had the stricturing phenotype as compared to patients without mutation (53% vs. 28%, p=0.00003). Double mutant patients had also less colonic involvement when compared to wild type patients (43% vs. 62%, p=0.003). When comparing age at onset between these two groups, double mutation carries had slightly lower age at onset (16.9 vs. 19.8 years, p=0.01).

Table 6. Rare potential disease-causing mutations of the *CARD15* gene in patients with Crohn's disease. CARD=caspase recruitment domain, NBD=nucleotide-binding domain, LRR=leucine-rich repeat.

Nucleotide change	Amino acid change	Protein domain	References
Exon 2			
G337A	D113N	CARD1	Tukel *et al.* 2004
G413A	R138Q	CARD2	Lesage *et al.* 2002
G418A	A140T	CARD2	Lesage *et al.* 2002
T469C	W157R	CARD2	Lesage *et al.* 2002
Exon 3			
C566T	T189M	CARD2	Lesage *et al.* 2002
Exon 4			
C703T	R235C		Lesage *et al.* 2002, King *et al.* 2006
T743G	L248R		Lesage *et al.* 2002, Tukel *et al.* 2004
A866G	N289S	NBD	Lesage *et al.* 2002, Schnitzler *et al.* 2006
G871A	D291N	NBD	Lesage *et al.* 2002
C931T	R311W	NBD	Lesage *et al.* 2002, Schnitzler *et al.* 2006
A1070C	D357A	NBD	Tukel *et al.* 2004
A1087T	I363F	NBD	Tukel *et al.* 2004
C1117T	R373C	NBD	Lesage *et al.* 2002
C1171T	R391C	NBD	Schnitzler *et al.* 2006
G1178A	R393H	NBD	King *et al.* 2006
A1241G	N414S	NBD	Lesage *et al.* 2002

C1292T	S431L	NBD	Lesage *et al.* 2002, King *et al.* 2006
G1321A	E441K	NBD	Lesage *et al.* 2002, Tukel *et al.* 2004
C1387G	P463A	NBD	Schnitzler *et al.* 2006
C1648G	L550V	NBD	Tukel *et al.* 2004
1671delCCTGGG	558delLG	NBD	Lesage *et al.* 2002
G1834A	A612T		Lesage *et al.* 2002, Tukel *et al.* 2004, King *et al.* 2006
C2003T	P668L		van Heel *et al.* 2006
C2050T	R684W		Lesage *et al.* 2002
C2107T	R703C		Lesage *et al.* 2002, Sun *et al.* 2003, Tukel *et al.* 2004, Lakatos *et al.* 2005, King *et al.* 2006, Schnitzler *et al.* 2006, van Heel *et al.* 2006
C2137T	R713C		Lesage *et al.* 2002, Lakatos *et al.* 2005
G2138A	R713H		Schnitzler *et al.* 2006
C2264T	A755V	LRR1	Lesage *et al.* 2002, Lakatos *et al.* 2005, Schnitzler *et al.* 2006, van Heel *et al.* 2006
C2278T	R760C	LRR1	Schnitzler *et al.* 2006
G2332A	E778K	LRR2	Lesage *et al.* 2002, Sun *et al.* 2003, Lakatos *et al.* 2005
C2368T	R790W	LRR2	Schnitzler *et al.* 2006
G2372A	R791Q	LRR2	Sugimura *et al.* 2003a, Lakatos *et al.* 2005
C2371T	R791W	LRR2	Schnitzler *et al.* 2006
G2377A	V793M	LRR2	Lesage *et al.* 2002, Sun *et al.* 2003, Lakatos *et al.* 2005, King *et al.* 2006
Exon 5			
C2475G	N825K	LRR4	Schnitzler *et al.* 2006
G2489A	R830Q	LRR4	King *et al.* 2006, van Heel *et al.* 2006
G2527A	E843K	LRR4	Lesage *et al.* 2002
C2546T	A849V	LRR4	Schnitzler *et al.* 2006
Exon 6			
A2555G	N852S	LRR4	Tukel *et al.* 2004, King *et al.* 2006
A2558G	N853S	LRR5	Lesage *et al.* 2002
A2587G	M863V	LRR5	Lesage *et al.* 2002, Tukel *et al.* 2004, King *et al.* 2006
Exon 7			
G2656A	A885T	LRR6	Lesage *et al.* 2002
C2686T	R896X	LRR6	King *et al.* 2006, van Heel *et al.* 2006

Exon 8			
C2753A	A918D	LRR7	Lesage *et al.* 2002, Tukel *et al.* 2004
G2771A	G924D	LRR7	Lesage *et al.* 2002
Intron 8			
IVS8+158C→T			Sugimura *et al.* 2003a
Exon 10			
G2929A	E977K	LRR9	van Heel *et al.* 2006
G2933A	G978E	LRR9	Lesage *et al.* 2002
Exon 12			
C3055G	R1019G	LRR10	King *et al.* 2006, van Heel *et al.* 2006

Pediatric CD patients (n=55) with average age of 11.2 at onset were studied for additional *CARD15* mutations and three potential disease-causing mutations were identified (R703C, E778K, and V793M, Table 6) (Sun *et al.* 2003). 67% of pediatric CD patients carried at least one of the three common mutations and the frequencies of other three missense mutations R703C, E778K, and V793M were 2% each. Nineteen (35%) patients were homozygous or compound heterozygous for the six disease-associated mutations and the 1007*fs* mutation was significantly more common in pediatric CD patients than in healthy controls (allele frequency 26% vs. 2%). Genotype-phenotype correlations showed a significant association between *CARD15* mutations and ileocolonic disease. Patients with two mutations had also more frequently undergone intestinal resection indicating possibility of more severe disease (=stricturing behaviour). All disease-associated alleles were in or close to the LRR domain which supports the finding of Lesage and co-workers (Lesage *et al.* 2002). The much higher frequency of double mutation carriers among the pediatric group also supports the idea of a gene-dosage effect for earlier age at onset.

In one study, 527 CD patients were screened for additional *CARD15* mutations in exon 4 in addition to the common mutations (Lakatos *et al.* 2005). As a result, five rare variants (R713C, A755V, E778K, R791Q, and V793M) were found and the R703C variant was identified as a risk factor for CD (2.1% vs. 0% controls, p=0.02) (Table 6). In other two studies, additional *CARD15* mutations were sought in a study population consisting of patients heterozygous for common *CARD15* mutations (King *et al.* 2006; Schnitzler *et al.* 2006). As a result of screening the coding region, 11 of 100 CD patients had a second potential disease-causing mutation (R235C, R393H, S431L, A612T, R703C, V793M, R830Q, N852S, M863V, R896X, and R1019G, Table 6) (King *et al.* 2006). In another study, nine rare potential disease-causing mutations were found, eight of them novel (R391C, P463A, R713H, R760C, R790W, R791W, N825K, and A849V) and one described previously (N289S) (Table 6) (Schnitzler *et al.* 2006). Rare variants R311W,

R703C, and A755V (Table 6) detected were considered as polymorphisms. A severe phenotype was observed in CD patients who were compound heterozygotes for the common and the novel *CARD15* mutation. In addition, a *CARD15* functional study reported an additional rare variant in seven heterozygous CD patients (P668L, R703C, A755V, R830Q, R896X, E977K, and R1019G, Table 6) (van Heel *et al.* 2006).

Although CD is more common among Jews (Roth *et al.* 1989), the frequency of the three common mutations are similar in Jews and Caucasians. Initial results using six microsatellite markers spanning the *IBD1* locus showed that Ashkenazi Jews might have additional mutations in this region (Sugimura *et al.* 2003a). Further evidence for additional variants was seen in a highly significant association between the SNP5 haplotype and Jewish CD patients. This led to the identification of two potential disease-associated alleles IVS8+158C→T and R791Q (Table 6). A haplotype carrying this intronic variant showed a higher risk for CD in Ashkenazi Jews than in non-Jews, even though the function of this haplotype remained unrecognised. In the second Jewish study, *CARD15* screening revealed eight potential disease-causing variants D113N, L248R, D357A, I363F, L550V, A612T, R703C, and N852S (Table 6) (Tukel *et al.* 2004).

Due to the absence of the three *CARD15* mutations in Japanese CD patients, screening of the whole coding area has been performed (Sugimura *et al.* 2003b). No amino acid change predisposing to CD was observed, further confirming that *CARD15* is not a contributor for CD susceptibility in Japan.

4.1.5 Functional consequences of *CARD15* mutations

NOD2-mediated functions identified to date include induction of pro- and anti-inflammatory cytokines and epithelial cell defences, up-regulation of costimulatory molecules leading to enhanced T cell activation, and mediation of antibacterial effect through different mechanisms (Cho and Abraham 2007). Defects in NOD2 function most likely contribute to some extent to alterations in intestinal immune homeostasis but how CD associated *CARD15* mutations increase the susceptibility to CD remains unknown. It has been shown that *CARD15* mutations lead to both gain and loss of function of this gene.

Transient transfection of cells has shown that the three common *CARD15* mutations result in defective recognition of MDP and this further leads to decreased NF-κB activation (Bonen *et al.* 2003; Chamaillard *et al.* 2003; Girardin *et al.* 2003; Inohara *et al.* 2003). In addition to three common *CARD15* mutations, multiple rare variants have been deficient

in their ability to sense MDP (Chamaillard *et al.* 2003). In another study, a comprehensive library of *CARD15* variants was generated resulting in 519 amino acid changes and their function was investigated in HEK293 cells (Tanabe *et al.* 2004). Essential regulatory domains and specific residues of *CARD15* involved in MDP recognition were identified. Amino acid residues 855-1040 in the LRR domain of the protein were found to be essential for the response to MDP.

A more sophisticated method for investigating NOD2 function has been the use of mononuclear cells derived from CD patients. In these studies the functional consequences of *CARD15* in response to MDP has been determined by the production of different cytokines. The 1007*fs* mutation results in defective release of anti-inflammatory cytokine IL-10 from blood mononuclear cells of CD patients after stimulation with the TLR2 ligands peptidoglycan and Pam3Cys-KKKK (Netea *et al.* 2004). In other study the 1007*fs* mutation was reported to exhibit defects in production of proinflammatory cytokines TNF-α, IL-6, and IL-8 (van Heel *et al.* 2005a). Compound heterozygotes of the three common mutations also showed reduced IL-8 production when compared to wild types. CD patients homozygous for the 1007*fs* mutation have also exhibited strongly decreased IL-1β synthesis in response to both PGN and MDP (Netea *et al.* 2005). Later it has been shown that healthy individuals heterozygous for the common *CARD15* mutation also have modest impairment of IL-8 response (van Heel *et al.* 2006). Macrophages from homozygous 1007*fs* patients have demonstrated a loss of NOD2-induced signalling in a microarray study (Li *et al.* 2004). There is now compelling evidence from primary cell studies to suggest that the common *CARD15* mutations lead to loss of function. However, this is inconsistent with the fact that CD is associated with overexpression of NF-κB target genes TNF-α, IL-1β, IL-6, and IL-12 under inflamed conditions (Monteleone *et al.* 1997; Parronchi *et al.* 1997; Rogler *et al.* 1998; Schreiber *et al.* 1998).

Studies of *CARD15* knockout mice have generated conflicting data as to whether mutations in this gene cause loss or gain of function (Watanabe *et al.* 2004; Kobayashi *et al.* 2005; Maeda *et al.* 2005). Using NOD2 knockout mice, Watanabe and colleagues showed that NOD2 signalling in normal circumstances inhibits the TLR-driven T helper 1 response by regulating NF-κB signalling (Watanabe *et al.* 2004). Thus, in the absence of NOD2 signalling there is no NOD2 inhibition and this results in increased TLR2-mediated NF-κB activation and even more IL-12 production. In another study, a mouse model of mutant NOD2 has exhibited elevated NF-κB activation in response to MDP and more efficient processing and secretion of the cytokine IL-1β (Maeda *et al.* 2005). However, in a third study on NOD2 knockout mice, protective immunity was abolished as a

consequence of loss of fuction of NOD2 (Kobayashi *et al.* 2005). It is still unclear why *CARD15* mutations seem to result in loss of function in humans and gain of function in mouse models and additional studies are needed to reconcile the differences between human and murine findings. NOD2 knockout mice do not spontaneously develop ileitis (Pauleau and Murray 2003; Kobayashi *et al.* 2005), demonstrating the requirement for other genetic factors for disease expression.

Studies on *CARD15* mutants, both in humans and mice, have demonstrated that normal gene function is required for optimal defensin expression (Wehkamp *et al.* 2004; Kobayashi *et al.* 2005; Wehkamp *et al.* 2005). Defensins are antimicrobial peptides which are synthesized and secreted by Paneth cells of the small intestine (Vignal *et al.* 2007). Antimicrobial peptides of the epithelia are known to kill a broad range of microorganisms, thus protecting the host from pathogens. Ileal expression of human α-defensin 5 (HD-5) and HD-6 is decreased in the affected ileum of CD patients and more importantly, a significant decrease in expression was observed in CD patients with *CARD15* mutations (Wehkamp *et al.* 2004; Wehkamp *et al.* 2005). Consistently, NOD2 knockout mice have a selective decrease in α-defensin expression (Kobayashi *et al.* 2005).

4.1.6 *CARD15* in Blau syndrome and early-onset sarcoidosis

The *CARD15* gene is also associated with Blau syndrome (BS; MIM 186580), a rare autosomal dominant disorder characterised by early-onset granulomatous arthritis, uveitis, and skin rash with camptodactyly (Blau 1985). Before identification of *CARD15* in BS susceptibility, the susceptibility locus had already been mapped to chromosome 16p12-q21 by linkage analysis (Tromp *et al.* 1996). Both CD and BS are associated with granulomatous inflammation but the disease-causing mutations in the *CARD15* gene are different. Four missense mutations (R334Q, R334W, E383K, and L469F) associated with BS are located in the central nucleotide-binding domain (NBD) (Miceli-Richard *et al.* 2001; van Duist *et al.* 2005) and exhibit enhanced basal NF-κB activity in contrast to CD associated mutations which cause decreased NF-κB activation *in vitro* (Chamaillard *et al.* 2003). However, a functional study on R334W mutation using patient mononuclear cells has shown normal responses to MDP (van Heel *et al.* 2006).

BS shows phenotypic overlap with early-onset sarcoidosis (EOS; MIM 609464). EOS is a rare disease usually starting before four years of age with a combination of skin, joint, and eye disorders (Kanazawa *et al.* 2005). EOS is also associated with mutations in the *CARD15* gene and the BS associated mutation R334W is also found in EOS (Kanazawa *et al.* 2004; Kanazawa *et al.* 2005; Coto-Segura *et al.* 2007). In addition, D382E, H496L,

M513T, T605P, A612T, and N670K missense mutations have been found in EOS patients and their functional consequences as well as the R334W mutation have been examined in transfected HEK293T cells (Kanazawa *et al.* 2005). All except the A612T variant significantly increased basal NF-κB activity when compared to the wild type. However, MDP-induced NF-κB activity was not greater for mutants than for the wild type.

4.2 The *DLG5* gene on chromosome 10

In 2004, a positional cloning approach led to identification of the discs large, drosophila, homolog of 5 (*DLG5*) gene (Table 5) (Stoll *et al.* 2004) after initial linkage of IBD to the pericentromeric region of chromosome 10 in a European cohort (Hampe *et al.* 1999a). DLG5 is a member of the MAGUK (membrane-associated gyanylate kinase) gene family which encodes scaffolding proteins involved in intracellular signal transduction (Gonzalez-Mariscal *et al.* 2000). DLG5, with multiple protein interaction domains, has also been implicated in maintaining epithelial integrity and polarity (Nakamura *et al.* 1998; Humbert *et al.* 2003). It is also widely expressed in human tissues as well as the GI tract (small intestine, intestinal epithelial cells, and colon) (Ferguson *et al.* 2007).

The association of genetic variations in the *DLG5* gene with IBD and particularly CD was initially described in German and UK study samples with two distinct haplotypes (Stoll *et al.* 2004). The haplotype A (protective), tagged by SNP DLG5_e26 ins/delA, was significantly undertransmitted in IBD and CD, whereas the haplotype D (risk), tagged by the SNP R30Q (G89A, previously reported as G113A), was significantly overtransmitted in these groups. The R30Q polymorphism is located in the DUF622 domain of the *DLG5* gene and is likely to have functional consequences. Further studies have, however, yielded inconsistent results (Table 5) (Yamazaki *et al.* 2004; Daly *et al.* 2005; Gazouli *et al.* 2005a; Noble *et al.* 2005b; Torok *et al.* 2005; Vermeire *et al.* 2005; Buning *et al.* 2006; Lakatos *et al.* 2006; Medici *et al.* 2006; Newman *et al.* 2006; Tremelling *et al.* 2006; Browning *et al.* 2007; Pearce *et al.* 2007; Russell *et al.* 2007), indicating that the effect appears to be inconsistent, relatively weak, and population specific (Russell *et al.* 2007). Phenotypic association between the rare variant 30Q and ileal CD has been reported (Pearce *et al.* 2007). Meta-analysis gave no evidence of association of 30Q with IBD indicating that this variant is not a risk factor for IBD across populations (Browning *et al.* 2007). In addition, two studies have reported that the rare variant is a risk factor for CD in men both in adults and children (Friedrichs *et al.* 2006; Russell *et al.* 2007) whereas one study on pediatric CD proposes that this variant has a protective effect in girls (Biank *et al.* 2007). The meta-analysis concerning the gender-stratified analysis from 11 published

studies showed that *DLG5* R30Q is associated with a small decrease in risk of CD in women (Browning *et al.* 2008).

4.3 The *ABCB1/MDR1* gene on chromosome 7

The ATP-binding cassette, sub-family B, member 1 (*ABCB1*) gene (also known as the *MDR1* gene) is located on chromosome 7q21, in a region of suggestive evidence for linkage for both CD and UC (Satsangi *et al.* 1996a; Cho *et al.* 1998; van Heel *et al.* 2004). *ABCB1* knockout mice also spontaneously develop colitis in a specific pathogen-free environment (Panwala *et al.* 1998). The condition can be prevented by and treated with antibiotics, suggesting that intestinal flora are necessary to initiate and maintain the inflammation (Annese *et al.* 2006). The *ABCB1* gene encodes P-glycoprotein 170, which functions as a transmembrane ATP-dependent efflux pump moving drugs and toxins from the intracellular to the extracellular domain (Annese *et al.* 2006). The protein is highly expressed at the apical surface of epithelia of the colon and distal small intestine (Thiebaut *et al.* 1987) and this suggests a role in protection not only against xenobiotics but also bacterial products (Ho *et al.* 2005). The protein has also been detected in haematopoietic stem cells, macrophages, dendritic cells, natural killer cells, and both T and B lymphocytes (Klimecki *et al.* 1994). *ABCB1* expression is significantly decreased in the colon of UC but not CD patients according to a recent finding (Langmann *et al.* 2004).

Two exonic polymorphisms, C3435T and G2677T/A (A893S/T), have received the most attention. Although the C3435T SNP does not change the amino acid, it has been shown to alter protein expression (Hoffmeyer *et al.* 2000). The other variant may also have an influence on protein expression (Kim *et al.* 2001; Tanabe *et al.* 2001). Association with CD and/or UC have been established in some, but not all, studied populations (Table 5) (Brant *et al.* 2003a; Croucher *et al.* 2003a; Schwab *et al.* 2003; Gazouli *et al.* 2004; Glas *et al.* 2004; Potocnik *et al.* 2004; Ho *et al.* 2005; Palmieri *et al.* 2005; Onnie *et al.* 2006b; Oostenbrug *et al.* 2006a; Osuga *et al.* 2006; Urcelay *et al.* 2006; Ardizzone *et al.* 2007; Cucchiara *et al.* 2007a; Farnood *et al.* 2007). A meta-analysis of nine published studies of the C3435T variant in IBD showed a significant association between the rare T allele and UC (Onnie *et al.* 2006b). In another meta-analysis, which partly employed the same published studies, an equivalent conclusion was made (Annese *et al.* 2006).

In addition, a gene-wide haplotype tagging strategy was recently conducted using six haplotype tagging SNPs (tSNPs) and showed a significant association especially with extensive UC which was critically dependent on one tSNP, an intronic variant rs3789243

(Ho *et al.* 2006). All haplotypes carrying this intronic variant maintained a highly significant association and the effect of this tSNP was independent of the C3435T variant.

4.4 The *TLR4* gene on chromosome 9

The innate immune response is the first line of defence against microbial infections. Together with the NLR family, the toll-like receptor (TLR) family proteins are in charge of the detection of different bacterial and viral components (Doyle and O'Neill 2006). These two protein families, known as pathogen-recognition receptors (PRRs), appear to play essential roles in mucosal homeostasis and alterations contributing to the pathogenesis of IBD (Yamamoto-Furusho and Podolsky 2007). Identification of *CARD15* as a CD susceptibility gene has raised the question whether other PRRs are involved in the pathogenesis of IBD. Toll-like receptor 4 (TLR4), in combination with CD14, LBP, and MD-2 proteins, acts as the PRR for lipopolysaccharide (LPS), a cell wall component of gram-negative bacteria (Aderem and Ulevitch 2000; Barton and Medzhitov 2003). Recognition of LPS triggers a cascade of cellular signals that culminate in NF-κB activation and, further, leads to proinflammatory cytokine production and clearance of the infectious agent (Figure 1) (Doyle and O'Neill 2006).

In mammals, the intestinal epithelium is a major barrier to the entry of microbes into an organism. TLR4 is expressed on the cell surface of antigen presenting cells including macrophages and dendritic cells as well as in the intestinal epithelium. Furthermore, alterations of TLR4 expression by intestinal epithelial cells have been described in IBD, showing that normally TLR4 is only barely detectable whereas in both UC and CD patients TLR4 is strongly upregulated (Cario and Podolsky 2000). Increased expression of TLR4 by myeloid dendritic cells in IBD has also been reported (Hart *et al.* 2005).

Two common polymorphisms (D299G and T399I) in the extracellular domain of the *TLR4* gene have been described in humans. The D299G polymorphism has been associated with decreased responsiveness followed by LPS stimulation (Arbour *et al.* 2000; Schwartz 2002; Michel *et al.* 2003) although recent data have shown a normal response (Erridge *et al.* 2003; Heesen *et al.* 2003; Imahara *et al.* 2005). The potential association between IBD and these two genetic variations in *TLR4* has been investigated by many groups (Table 5) (Arnott *et al.* 2004; Franchimont *et al.* 2004; Torok *et al.* 2004; Braat *et al.* 2005; Brand *et al.* 2005; Gazouli *et al.* 2005b; Lakatos *et al.* 2005; Oostenbrug *et al.* 2005; Ouburg *et al.* 2005; Riis *et al.* 2007). The results have been contradictory, which has been explained to be due to either population-related genetic heterogeneity, stratification bias, or sample size (Arnott *et al.* 2005). The most recent study, combining results of its own and eight

published reports concerning the D299G variant and a total of 4805 IBD patients, showed an association with IBD and also with CD (De Jager *et al.* 2007).

4.5 The *TRAF6* gene on chromosome 11

Regulation of intestinal innate immunity involves cell surface and intracellular PRRs, adapter proteins, kinases, and the NF-kB system. Tumor necrosis factor (TNF) receptor-associated factors constitute a family of signal transducers that are thought to play important functions in innate and adaptive immunity, inflammation, and tissue homeostasis (Wu and Arron 2003). A member of this family, TRAF6, is an adaptor protein in the transcription factor NF-kB and activator protein-1 (AP-1) pathways mediating signalling downstream from receptors of the TNFR superfamily (though not TNFRSF1A) and TLR/IL-1R family (including TLR4) (Figure 1) (Cao *et al.* 1996; Deng *et al.* 2000; Ye *et al.* 2002; Wu and Arron 2003). While NF-kB is known to promote expression of genes involved in inflammatory responses and protection from apoptosis (Beg and Baltimore 1996), the stimulation of AP-1 activity may elicit stress responses and promote both cell survival and death (Shaulian and Karin 2001). In addition, the most recent findings suggest that NOD2 activation can cause the activation of TRAF6, making TRAF6 a common factor in two PRR pathways (Figure 1) (Abbott *et al.* 2007). Interestingly, one of the nominal linkages in the Finnish IBD scan was localised to chromosomal region 11p12-q13, where *TRAF6* is also located (Paavola-Sakki *et al.* 2003).

4.6 The *TNFRSF1A* gene on chromosome 12

Tumor necrosis factor-alpha (TNFα) is a proinflammatory cytokine mediating apoptosis, inflammation, and immunity, and has been implicated in the pathogenesis of several human diseases, including IBD (Chen and Goeddel 2002). TNFα is secreted primarily by activated monocytes, macrophages, and T cells where activation of TLRs by a variety of PAMPs or cytokine receptors such as IL-1R stimulates transcription factor NF-κB that increases TNFα expression (Shealy and Visvanathan 2008). TNFα, in turn, signals through two distinct cell surface receptors TNFRSF1A and TNFRSF1B. TNF receptor superfamily member 1A (*TNFRSF1A*) (also known as TNFR1 and p55) is expressed on virtually all nucleated cells while the expression of TNF receptor superfamily member 1B (*TNFRSF1B*) is limited to immune and endothelial cells (Aggarwal 2003). TNFα/TNF receptor interactions play an essential role in the pathogenesis of the inflammatory response.

It has been shown that TNFα levels are elevated in serum, stool, and inflamed bowel mucosa of IBD patients (Braegger *et al.* 1992; Murch *et al.* 1993; Nicholls *et al.* 1993; Breese *et al.* 1994; Komatsu *et al.* 2001; Borruel *et al.* 2002). Several animal models have provided the foundation for the development of anti-TNF antibodies as treatment for chronic inflammatory diseases. IgG1 monoclonal antibodies against TNFα are an important advance in the treatment of CD and lately also of UC. The goal of this treatment is to reduce TNFα levels in the circulation to improve the clinical signs of the disease without causing systemic immunosuppression (Shealy and Visvanathan 2008).

Given the importance of TNF-receptor mediated signalling pathway in IBD and, in addition, that the *TNFRSF1A* gene is located near the *IBD2* locus, the association of *TNFRSF1A* to IBD has also been studied (Table 5). In the first such study, an association between the A36G variant of the *TNFRSF1A* gene and pancolitis in UC was observed (Pierik *et al.* 2004). In another study, there was an association of the same *TNFRSF1A* gene variant with CD (Waschke *et al.* 2005). The same variant was negatively associated with the stricturing CD phenotype. However, in Japan no association between the A36G variant and IBD has been reported (Sashio *et al.* 2002).

4.7 The *IL23R* gene on chromosome 1

Recently, a highly significant association between ileal CD and the *IL23R* gene on chromosome 1p31 was reported (Table 4) (Duerr *et al.* 2006). In this genome-wide association study over 300 000 SNP markers were typed in a North American ileal CD case-control material. The most significant associations were observed to the CD susceptibility gene *CARD15* and to the *IL23R* gene. A nonsynonymous *IL23R* SNP (R381Q) conferred a strong protection against CD. Nine additional noncoding markers at this locus were also associated. In the same study, replication analyses on a Jewish case-control cohort as well as 883 nuclear families were positive. The latter analysis also showed association with non-Jewish UC. Three subsequent genome-wide association studies have also identified the *IL23R* gene in CD susceptibility (Consortium 2007; Libioulle *et al.* 2007; Raelson *et al.* 2007).

Several replication studies on *IL23R* have already been conducted and association with both CD and UC has been reported (Table 5) (Borgiani *et al.* 2007; Buning *et al.* 2007; Cummings *et al.* 2007a; Glas *et al.* 2007; Oliver *et al.* 2007; Roberts *et al.* 2007; Tremelling *et al.* 2007; Weersma *et al.* 2008). The protective R381Q allele has been reported in pediatric CD (Baldassano *et al.* 2007b; Dubinsky *et al.* 2007) and IBD (Van

Limbergen *et al.* 2007a) as well. The only negative association was with Japanese CD patients (Yamazaki *et al.* 2007).

The IL23R protein was identified and characterised in 2002 (Parham *et al.* 2002). The functional IL23R protein is heterodimeric, comprising the *IL23R* subunit on chromosome 1p31 and an *IL12Rβ1* subunit on chromosome 19p13. The latter subunit is also common to the IL12 receptor which has an *IL12Rβ2* subunit as the second part. The ligand, a proinflammatory cytokine interleukin 23 (IL-23), is also heterodimeric and is composed of a unique p19 subunit (chromosome 12q13) and p40 subunit (chromosome 5q33), which is also a component of IL-12 (Oppmann *et al.* 2000). The other component of IL-12 is the p35 subunit. IL-23 is a pivotal cytokine in the differentiation of T helper cells, especially their differentiation into Th17 T cells (Neurath 2007; Steinman 2007). In IBD, the well controlled balance of the intestinal immune system is disturbed and myeloid dendritic cells falsely recognise commensal bacteria as pathogens (Baumgart and Carding 2007). This leads to maturation of dendritic cells and they further promote differentiation of naive T cells (Th0) into effector Th1, Th2, and Th17 cells as well as natural killer T cells. Traditionally, it has been thought that CD is driven through Th1 cell response and UC through Th2 cells (Baumgart and Carding 2007). Th17 cells on the other hand are different from classic Th1 and Th2 cells, both in the cytokines that induce their differentiation and the cytokines they produce (Cho and Weaver 2007). Clear evidence exists now for the central role of IL-23 in the development of CD.

4.8 The *ATG16L1* gene on chromosome 2

In the European GWA study of nearly 20 000 nonsynonymous SNPs, an amino acid substitution (T300A) within the autophagy related 16-like 1 (*ATG16L1*) gene was found to be highly associated with CD (Table 4) (Hampe *et al.* 2007). Haplotype and regression analyses showed that the coding variant T300A carried virtually all the disease risk for CD exerted by this locus. Soon after that, three other GWA studies reported the same finding (Consortium 2007; Libioulle *et al.* 2007; Rioux *et al.* 2007) and several replication studies have also confirmed the association with CD (Table 5) (Baldassano *et al.* 2007a; Cummings *et al.* 2007b; Prescott *et al.* 2007; Roberts *et al.* 2007; Glas *et al.* 2008; Van Limbergen *et al.* 2008; Weersma *et al.* 2008). Two studies have reported phenotypic association of the *ATG16L1* gene variation with ileal CD (Prescott *et al.* 2007; Van Limbergen *et al.* 2008). A modest association with UC has also been demonstrated in one study (Prescott *et al.* 2007).

The ATG16L1 protein is involved in the autophagocytosis pathway, which is known to account for degradation of long-lived proteins and organelles and in addition to mediate resistance to intracellular pathogens such as bacteria and viral particles (Schmid *et al.* 2006). Autophagic degradation links innate with adaptive immunity by delivering antigens to MHC class II presentation. ATG16L1 is one component of a large protein complex essential for autophagy (Mizushima *et al.* 2003). Expression of *ATG16L1* has been detected in various tissues including the colon, small intestine, intestinal epithelial cells, leukocytes (T and B cells), and spleen (Hampe *et al.* 2007; Rioux *et al.* 2007). RNA-silencing methods have been used to knock-down the expression of the gene in human HeLa cells, and this decreased autophagy of *Salmonella typhimurium* (Rioux *et al.* 2007).

5. STUDIES OF SPECIFIC GENE LOCI

5.1 Chromosome 5 risk haplotype (*IBD5* locus)

The *IBD5* locus on chromosome 5 was originally identified in 1999 (Table 3) (Ma *et al.* 1999). This finding was replicated by higher-density mapping a year later in a Canadian CD cohort (Rioux *et al.* 2000) and the same authors further narrowed the associated area to chromosome 5q31 (Rioux *et al.* 2001). In this region a 250 kilobase (kb) haplotype shows strong association with CD. The haplotype contains 11 haplotype tagging SNPs equally associating with CD which means strong linkage disequilibrium (LD) across this region. The region of association also contains a relatively gene-rich area including the cytokine gene cluster. Since the identification of this risk haplotype, several groups have independently confirmed the association between this haplotype and CD (Armuzzi *et al.* 2003; Giallourakis *et al.* 2003; Mirza *et al.* 2003; Negoro *et al.* 2003; Latiano *et al.* 2006; Torkvist *et al.* 2007). In some studies association with UC has also been established (Giallourakis *et al.* 2003; McGovern *et al.* 2003).

In 2004, two functional polymorphisms 1672C→T and -207G→C within the *SLC22A4* (OCTN1) and *SLC22A5* (OCTN2) genes, respectively, were proposed to be the critical mutations associating with CD susceptibility in this region (Table 5) (Peltekova *et al.* 2004). These two polymorphisms were in strong LD, creating a 2-allele risk haplotype (SLC22A-TC) which was enriched among CD patients. Preliminary functional studies of these variants demonstrated altered transcription and transporter activity of various organic cations. After this finding several replication studies in different populations have been performed with conflicting results. Positive association with CD has been obtained in several populations (Table 5) (Gazouli *et al.* 2005a; Noble *et al.* 2005a; Torok *et al.* 2005; Fisher *et al.* 2006; Leung *et al.* 2006; Martinez *et al.* 2006; Onnie *et al.* 2006a; Torkvist *et*

al. 2007) and also with IBD in one study (Waller *et al.* 2006). Many studies have also reported that this association is dependent on the background risk haplotype (Noble *et al.* 2005a; Fisher *et al.* 2006; Onnie *et al.* 2006a; Russell *et al.* 2006; Waller *et al.* 2006; Torkvist *et al.* 2007). Two studies have reported association between *SLC22A* variants and pediatric CD patients from North America (Babusukumar *et al.* 2006) and Italy (Cucchiara *et al.* 2007b) and one with pediatric IBD (Russell *et al.* 2006). Many studies have resulted in negative association, including for Japanese (Yamazaki *et al.* 2004; Vermeire *et al.* 2005) and Flemish (Vermeire *et al.* 2005) patients.

Although there is controversy over the true causal gene within the *IBD5* locus, studies have tried to further assess the specific phenotype associated with this region. As in the case of true variants, findings related to the associated phenotype are inconsistent. Several groups have reported a lack of association between *IBD5* and a specific disease location whereas some have reported an association to perianal (Armuzzi *et al.* 2003; Vermeire *et al.* 2005), ileocolonic (Gazouli *et al.* 2005a; Leung *et al.* 2006), or colonic involvement (Gazouli *et al.* 2005a). In addition, penetrating (Urcelay *et al.* 2005; Vermeire *et al.* 2005; Latiano *et al.* 2006) and stricturing (Latiano *et al.* 2006) phenotype associations have been reported with this locus.

Even though the association of the *IBD5* locus to CD has been replicated repeatedly, the precise causal variants remain unknown, most likely due to strong LD across this region. Recently, the *SLC22A5* gene variant was excluded as the potential causal variant (Silverberg *et al.* 2007). During the year 2007, GWA studies in CD have also been able to replicate the *IBD5* locus and this even further strengthens the association with CD (Consortium 2007; Hampe *et al.* 2007; Raelson *et al.* 2007).

5.2 Major histocompatibility complex region (*IBD3* locus)

The major histocompatibility complex (MHC) on chromosome 6p21.3 has been the most studied genetic region in IBD (Ahmad *et al.* 2006). The MHC region, known in humans as the human leukocyte antigen (HLA) region, spans approximately four megabases (Mb) and contains a few hundred genes (Consortium 1999; Yap *et al.* 2004). The first report of HLA association with IBD was published in 1972 (Gleeson *et al.* 1972) and since then more than 100 studies have replicated the HLA association in IBD susceptibility. The *IBD3* locus on chromosome 6p includes the MHC region and has been linked to both UC and CD susceptibility.

Several independent genome-wide linkage studies have also shown evidence of linkage to *IBD3* (Tale 3). The first linkage to chromosome 6 containing the HLA region with the IBD phenotype was reported in 1999 (Hampe *et al.* 1999a) and soon after linkage to CD was also established (Ma *et al.* 1999). Since then, several other studies have confirmed the linkage to 6p (Hampe *et al.* 1999b; Yang *et al.* 1999b; Rioux *et al.* 2000; Dechairo *et al.* 2001; Barmada *et al.* 2004). This region was further underlined by a meta-analysis of ten published genome scans (van Heel *et al.* 2004). The study included 1952 IBD affected relative pairs, and *IBD3* was the only locus that gained genome-wide significance. It is also noteworthy that this locus presented stronger evidence of linkage than the *IBD1* locus, which contains the CD susceptibility gene *CARD15*. However, more recent GWA studies have not confirmed association with this region. Lack of association is most likely due to the use of CD rather than UC patient material.

The MHC is divided into three different regions: HLA class II (centromeric), class III, and class I (telomeric) (Consortium 1999). Numerous case-control studies in IBD have evaluated association with the classical class I and II alleles because they play a central role in the human immune response. Although many of these associations have been conflicting, association to IBD appear to be with class II rather than class I genes. The classical HLA class II genes encode cell-surface glycoproteins expressed on antigen presenting cells (APC) (Ahmad *et al.* 2006). The major role of these glycoproteins is to present different peptides (antigens) to T cell receptors (TCR) which in turn results in helper T cell ($CD4^+T$) activation. There are three types of HLA class II molecules; HLA-DR, HLA-DQ, and HLA-DP. Each of these molecules is made of αβ heterodimers ($\alpha_1\alpha_2\beta_1\beta_2$). The α chains are encoded by DRA, DQA, and DPQ genes. The first is invariant while the other two are highly polymorphic. The β chains of DRB1, DQB1, and DPB1 are also highly polymorphic. Gene duplication events have occurred in the past producing additional DRB3, 4, and 5 genes and these are expressed at low levels on the cell surface (Yap *et al.* 2004). Of the HLA class II molecules, HLA-DRB1 is the most extensively studied gene in IBD (Ahmad *et al.* 2006).

5.2.2 The HLA-DRB1 gene association with IBD

The HLA-DRB1 gene is the most polymorphic of the HLA-DR molecules and is present in all individuals (Stokkers *et al.* 1999; Annese *et al.* 2005b). This gene has almost 200 alleles. The most reliable HLA-DRB1 associations were highlighted in a meta-analysis of 29 studies (Stokkers *et al.* 1999), where significant positive association was achieved in UC with the DRB1*0103 and DRB1*1502 alleles. In CD, positive association was found for the DRB1*0401 and DRB1*0701 alleles.

The HLA-DRB1*0103 allele encodes an HLA-DR molecule with a unique sequence within peptide-binding groove, allowing it to bind and present potentially disease-associated peptides (Coppin *et al.* 1993). It is a rare allele with less than 2% frequency in Caucasian populations but still this is the most replicated HLA class II allele in UC susceptibility (Ahmad *et al.* 2006). It is also present at similar frequency in the Jewish population but absent from the Japanese, the same phenomenon observed with *CARD15* mutation frequencies. A strong association between this rare allele and UC was introduced for the first time in 1996 (Satsangi *et al.* 1996b), and a few years later also with CD (Trachtenberg *et al.* 2000). In the meta-analysis, three substudies also showed a moderate association in unselected UC patients (Stokkers *et al.* 1999). The association of this rare allele with UC has been later confirmed in different populations, namely in Dutch (Bouma *et al.* 1999), Spanish (de la Concha *et al.* 1999), North American (Trachtenberg *et al.* 2000; Silverberg *et al.* 2003), British (Ahmad *et al.* 2003), and Mexican (Yamamoto-Furusho *et al.* 2003) cohorts. In the first study, association with the rare allele was strongest among male patients with extensive disease (Satsangi *et al.* 1996b). This allele was also associated with severe disease requiring colectomy. In another study, this same allele was found more frequently in UC patients who underwent the surgery (Roussomoustakaki *et al.* 1997). Extensive disease involvement and extraintestinal manifestations were also more common among DRB1*0103 allele carriers. Other studies have further confirmed both extensive colitis and severe disease as defined by a need for (procto)colectomy among DRB1*0103 positive UC patients (Bouma *et al.* 1999; Ahmad *et al.* 2003; Yamamoto-Furusho *et al.* 2003). This uncommon allele may also be associated with a shorter mean time for surgery among patients who require colectomy (Ahmad *et al.* 2003).

CD is also associated with the DRB1*0103 allele in North American (Trachtenberg *et al.* 2000; Silverberg *et al.* 2003; Newman *et al.* 2004) and Spanish populations (Fernandez *et al.* 2004). The frequency of the DRB1*0103 allele was 10 fold higher in CD patients with colonic involvement than in healthy controls (Silverberg *et al.* 2003). This association has been replicated later in other studies (Fernandez *et al.* 2004; Newman *et al.* 2004). Perianal involvement and penetrating behaviour have also been shown to associate with this uncommon allele in two studies (Ahmad *et al.* 2002; Fernandez *et al.* 2004). It is quite clear now that this rare DRB1*0103 allele is a risk factor for both UC and colonic CD, suggesting it may have a role in chronic inflammation of the colon which is independent of UC or CD.

AIMS OF THE STUDY

The aims of the present study were to search for genes and their alterations predisposing to Crohn's disease and ulcerative colitis, the two major forms of inflammatory bowel diseases, to define genotype-phenotype relationships in IBD, and to delineate functional consequences of the genetic variations detected.

The specific aims were to

1. evaluate the allele frequencies of the common *CARD15* mutations in Finnish IBD patients, and search for possible associations between genotype and phenotype.
2. search for novel *CARD15* mutations in Finnish CD patients, to assess their functional consequences, and to relate their presence with the clinical phenotype.
3. participate into an international collaboration aimed at evaluating the allele frequencies and disease-related risks of the common *CARD15* mutations in the population.
4. identify variants of other candidate genes that are associated with risk or phenotypes of IBD.

PATIENTS AND METHODS

1. STUDY SUBJECTS

1.1 Patients with inflammatory bowel disease

Consecutive IBD patients treated at the departments of Gastroenterology and Surgery of the Helsinki University Hospital and Maria Hospital (a municipal hospital of Helsinki) during the years 1995-1998 or consecutive UC patients who had undergone colectomy in the 1990s at the Helsinki University Hospital were asked to participate in the study (Halme *et al.* 2002). Familial patients were also recruited from other parts of the country with the assistance of colleagues from the other University Hospitals in Finland. The diagnosis of CD and UC was based on standard endoscopical and histological criteria (Lennard-Jones 1989). A questionnaire on the history and details of their disease as well as the number of their affected and non-affected relatives were given to 1000 patients; 82% of the patients returned the questionnaire and gave their consent to the study. Patients were considered as familial IBD cases if they had at least one affected first-degree family member. In families with two or more affected members, the first member returning the questionnaire was termed the proband. Patients reporting parents originating outside Finland and affected relatives more distant than first-degree relatives were excluded. The final material of the present study consisted of 754 IBD patients, 459 of them being UC and 295 CD patients (Table 7).

Table 7. Number of patients and healthy controls used in studies I-V.

Study	***CD***		***UC***		***Mixed families***		***Controls***
	Familial	Sporadic	Familial	Sporadic	CD	UC	
I	46	198	37	62	27	-	300
II	44	196	-	-	-	-	190+6*
III	-	-	-	-	-	-	3575
IV							
initial cohort	20	-	39	-	36	-	320
extended cohort	44	195	78	338	51	-	-
V	44	196	115	344	-	-	190-312

*190 blood donors and 6 healthy individuals from laboratory staff

Details on the course of the disease were collected from the patient records. The location of inflammation was defined according to the largest macro- and microscopic extent registered at some time during the history of the disease. Location of inflammation in UC was classified as either left-sided or extended. For classifying the severity of the disease,

the need for medication and surgery was taken into account. CD was graded according to the Vienna classification (Gasche *et al.* 2000).

1.2 Healthy controls

DNA samples from anonymous healthy blood donors served as controls for the estimation of the allele frequencies in the background population. In studies I-V, varying numbers of blood donors were used as controls (Table 7). The samples were kindly provided by Dr. Tom Krusius, the Finnish Red Cross Blood Transfusion Service, Helsinki, Finland. Blood donors were from the capital city area of Helsinki. Mean age of the blood donors was 45 years at blood sampling, with equal numbers of women and men.

In study III, 3575 healthy individuals of Caucasian origin recruited by 15 groups from three different continents were studied for the three common *CARD15* mutations. Healthy individuals were a mixture of blood donors, healthy spouses of affected patients, and control groups from other studies. Only information on their European ancestry and apparent health was available for all cohorts. The number of healthy controls in different cohorts varied between 45 and 587. The Finnish cohort consisted of 300 blood donors. For functional *in vitro* studies in study II, six volunteers from the laboratory staff were used as healthy controls.

1.3 Ethical aspects

Informed written consent was obtained from all study subjects. In addition, the local Ethics Review Committee of the Department of Medicine, Helsinki University Hospital, had approved these studies.

2. METHODS

The methods used in the present study are described in more detail in the original publications I to V. They are also combined into Table 8.

Table 8. Methods used in the original publications in this thesis. The numbers of the original publications are indicated by their Roman numerals I-V.

Method	**Publication**
DNA extraction	I, II, III, IV, V
PCR	I, II, III, IV, V
RFLP	I, II, III, V
dHPLC	II, IV
Sequencing/Pyrosequencing	II, III, IV
Real time quantitative PCR	III
MassARRAY by Sequenom	III, V
Recombinant DNA techniques (cloning)	IV
Functional studies *in vitro*	II
ELISA	II
Bioinformatic analysis	I, II, IV, V
Statistical analysis	I, II, III, IV, V

RESULTS

1. MUTATIONS IN THE *CARD15* GENE AND THEIR RELATION TO CLINICAL PHENOTYPES

1.1 The *CARD15* gene mutation spectrum (I, II)

When this study was initiated, the association of the *CARD15* gene with CD was already established. Three mutations, R702W, G908R, and 1007*fs*, in the *CARD15* gene responsible for CD susceptibility were investigated in the Finnish cohort of 271 CD patients, 99 UC patients, and 300 blood donors as background population. CD patients were from three different categories based on their family relationships. Forty-six of these patients were familial cases and 198 were without family history of CD. The remaining 27 CD patients were from mixed IBD families. Results showed that 15.5% of CD patients, 9.1% of UC patients, and 6.7% of controls carried at least one of the three *CARD15* mutations. Allele frequencies of the common mutations are summarised in Table 9. The allele frequency of the 1007*fs* mutation was significantly higher in CD patients than in healthy blood donors (4.8% vs. 1.7%, $p<0.01$) and the corresponding frequency in familial probands was also statistically different when compared to sporadic patients (10.9% vs. 3.5%, $p<0.01$). The combined frequency of the three mutations between CD patients and controls was also significantly different (8.7% vs. 3.5%, $p<0.01$). One sporadic and two familial CD patients were homozygous for the 1007*fs* mutation and their affected siblings were homozygotes as well. Three compound heterozygotes were also identified, two of them being CD patients with the R702W and G908R mutations and one control carrying the R702W and 1007*fs* mutations.

Table 9. Allele frequencies of the three common *CARD15* mutations in patients with CD and UC and in blood donors.

Group	R702W	G908R	1007*fs*	Together
CD sporadic (n=198)	0.033	0.005	0.035	0.073
CD familial (n=46)	0.033	0.011	0.109	0.153
CD mixed families (n=27)	0.037	0.000	0.037	0.074
CD all (n=271)	0.033	0.006	0.048	0.087
UC (n=99)	0.015	0.000	0.030	0.045
Blood donors (n=300)	0.018	0.000	0.017	0.035

In the subsequent study (study II), a total of 240 unrelated patients with CD were screened for additional mutations in the *CARD15* gene by dHPLC and direct sequencing. A total of 30 different sequence variants were identified of which 25 were in the exonic and 5 in the

untranslated or intronic regions (Table 10). Eighteen of 25 exonic variants were predicted to result in amino acid changes. Thirteen of these amino acid substitutions, including the three common mutations, were previously described, while five were novel.

Of the sequence variants occurring in more than a single individual, only the frequency of the 1007*fs* mutation was significantly higher in patients (4.8%) than in controls (1.6%, p=0.01) in study II. The allele frequencies of the R702W and G908R mutations in CD patients were similar to those in study I (3.3% and 0.4%, respectively) and the corresponding frequencies in controls were 1.6% and 0%, respectively. There was no significant association of these two mutations with the risk of CD in accordance with previous results (study I).

The five novel amino acid substitutions R38M, W355X, P727L, W907R, and R1019X each occurred in a sporadic CD patient but in none of the healthy blood donors (Figure 2). Two of these mutations were nonsense (W355X and R1019X) and the remaining three were missense mutations (R38M, P727L, and W907R). The W355X mutation co-occurred with the 1007*fs* mutation, while the R1019X mutation occurred alone. The patient with the R38M mutation was also a homozygote for the 1007*fs* mutation, indicating that the R38M mutation had once occurred in the allele containing the 1007*fs* alteration. Furthermore, the patient carrying the P727L variant was also carrying the 1007*fs* mutation; again, these two mutations were on the same chromosome as confirmed by DNA analysis of the patient's daughter. The third patient with the W907R variant was also heterozygous for the T294S variant.

The previously described amino acid changes T189M, N289S, R791Q, and M863V were each found in one individual patient but none of the controls. It is again of note that the patient with N289S as well as the patient with M863V was a carrier of the common 1007*fs* mutation, but the allelic relation of these variants could not be determined. The remaining five amino acid substitutions were absent in CD patients (A660G) or occurred in similar frequencies in patient and control groups (T294S, R703C, A755V, and V955I), suggesting that they may not be related to CD susceptibility.

Seven nucleotide changes (C534G, A621T, C1377T, T1761G, C1833T, G2406T, and C2862T) which did not change amino acids were also found (Table 10). Their allele frequencies did not differ between patients and controls, and thus these variations were considered as silent polymorphisms. Five noncoding sequence variants were also identified, four of them in the intervening sequences (IVS2-29T→G, IVS2-25G→T,

IVS4+10A→C, and IVS7-15delCT) and one in the 5' untranslated region (5'UTR-59G→A). There was no difference in their frequencies between patients and controls.

Table 10. *CARD15* mutations and polymorphisms identified in patients with CD and the background population. Novel amino acid changes are shown in bold. CARD=caspase recruitment domain, NBD=nucleotide-binding domain, LRR=leucine-rich repeat.

Nucleotide change	Amino acid change	Protein domain	CD patients (n=250)	Healthy controls (n=190)
-59G>A			99	79
-29T>G			18	9
-25G>T			104	78
G113T	**R38M**	CARD1	1	0
C534G	S178S	CARD2	195	151
C566T	T189M	CARD2	1	0
A621T	P207P	CARD2	0	1
C802T	P268S		98	63
A866G	N289S	NBD	1	0
C881G	T294S	NBD	6	5
G1065A	**W355X**	NBD	1	0
C1377T	R459R	NBD	104	63
T1761G	R587R		185	145
C1833T	A611A		4	8
C1979G	A660G		0	1
C2104T	R702W		16	6
C2107T	R703C		2	1
C2180T	**P727L**		1	0
C2264T	A755V	LRR1	6	2
G2372A	R791Q	LRR2	1	0
G2406T	V802V	LRR2	3	1
IVS4+10A>C			26	20
A2587G	M863V	LRR5	1	0
IVS7-15delCT			5	4
T2719C	**W907R**	LRR6	1	0
G2722C	G908R	LRR6	2	0
C2862T	N954N	LRR8	0	1
G2863A	V955I	LRR8	25	13
3020*ins*C	1007*fs*	LRR10	23	6
C3055T	**R1019X**	LRR10	1	0

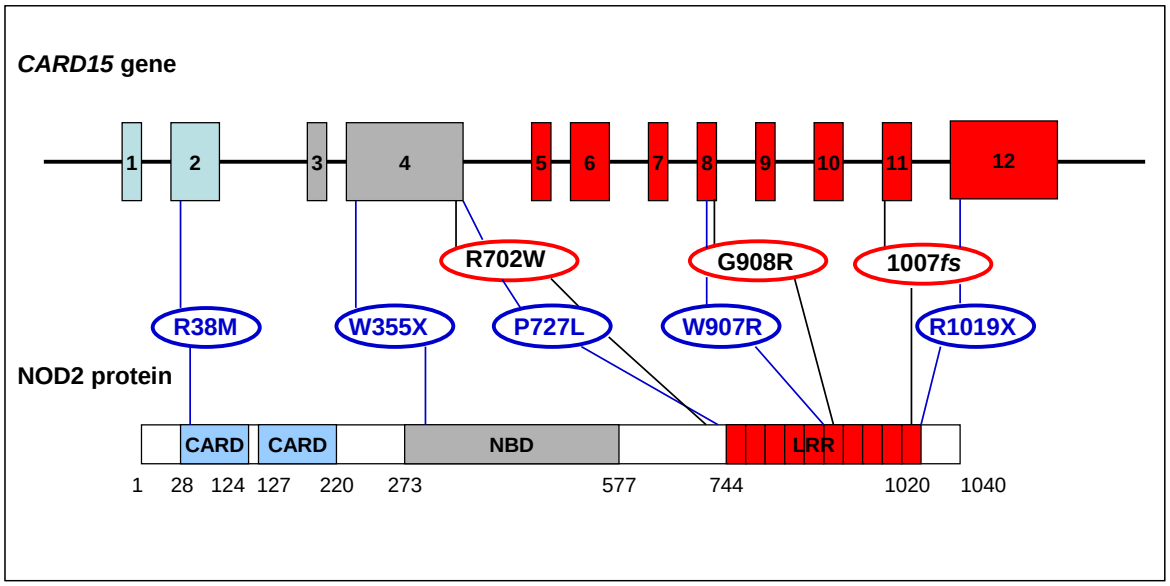

Figure 2. Gene and protein structure of CARD15. The three common mutations of the *CARD15* gene are shown here with red circles. Novel amino acid substitutions are indicated in blue.

1.2 The *CARD15* mutation frequency in the background population (III)

The allele and genotype frequencies were estimated for the R702W, G908R, and 1007*fs* mutations in 3575 healthy people of Caucasian origin. This background population was obtained from 15 different groups in Europe, North America, and Australia. The combined allele frequencies of the R702W, G908R, and 1007*fs* mutations were 4.3%, 1.2%, and 2.3%, respectively (Table 11), with large geographic fluctuations of the G908R, 1007*fs*, and wild type alleles ($p<0.0001$). Comparison of individual cohorts showed that the G908R mutation was absent in Finland and also very rare in Scotland but frequent in Italy and California (Table 11). The 1007*fs* mutation was in contrast rare in Italy and Brisbane but it was frequently found in Cambridge as well as in Germany and Chicago (Table 11). Comparison of wild type allele frequencies showed that *CARD15* mutations were rare in Finland and Brisbane, while they were common in Belgium and Cambridge (Table 11).

Among 3575 healthy controls, 18 were double mutation carriers. According to Hardy-Weinberg equilibrium, the expected number in this cohort was 23 but this deviation was not statistically significant. This provided strong evidence that the penetrance of the common mutations is low. Finally, in a case of Mendelian inheritance with limited heterogeneity, the disease incidence is expected to be directly related to mutation frequency. Thus, the relationship between the annual incidence and the *CARD15* mutation frequency in different populations were explored. No simple relationship was observed between these parameters for the cohorts studied.

Table 11. Minor allele frequencies of the three common *CARD15* mutations in apparently healthy individuals across three continents.

Population	Controls (n)	R702W	G908R	1007*fs*	Total
Finland	300	0.018	0.000	0.017	0.035
Scotland	136	0.048	0.004	0.018	0.070
England (Oxford)	587	0.042	0.011	0.019	0.072
England (Cambridge)	351	0.057	0.010	0.041	0.108
Belgium	144	0.066	0.021	0.028	0.115
Germany	370	0.045	0.007	0.038	0.090
Italy	205	0.041	0.027	0.007	0.075
France	174	0.049	0.011	0.026	0.086
Canada (Quebec)	58	0.052	0.009	0.009	0.070
Canada (Toronto)	100	0.050	0.020	0.030	0.100
USA (Chicago)	273	0.037	0.016	0.038	0.091
USA (California)	220	0.036	0.030	0.016	0.082
USA (Mid-Atlantic)	45	0.033	0.011	0.033	0.077
Australia (Brisbane)	203	0.039	0.007	0.007	0.053
Australia (Canberra)	409	0.045	0.007	0.010	0.062
Total	3575	0.043	0.012	0.023	0.078

1.3 Phenotypic characteristics of *CARD15* mutations (I, II)

The phenotypic characteristics of the CD patients were assessed according to the Vienna classification (Table 2) (Gasche *et al.* 2000). In addition, patient gender, familial status, duration of the disease, medication, indication for surgery, and smoking behaviour were investigated. Upon analysis of the three common mutations, CD patients with one or two mutations more often had ileal disease location (90% vs. 73%, $p<0.05$). In addition, these patients also more often had a complicated form of the disease as indicated by stricturing and penetrating behaviour (88% vs. 56%, $p<0.01$). Median disease duration was also two times longer among mutation carriers than non-carriers (12.3 vs. 6.0 years, $p<0.01$); however, upon comparison of ages at diagnoses no significant difference was seen. The need for bowel surgery was more common among mutation carriers than among non-carriers (67% vs. 46%, $p<0.05$).

Two familial CD patients and their affected siblings were homozygous for the 1007*fs* mutation. These four CD patients shared some phenotypic characteristics, including young age at diagnosis (≤20 years), an ileal location, and stricturing behaviour of the disease. One single sporadic CD patient who was homozygous for the 1007*fs* mutation also had the right colon affected in addition to the ileum. In this case, the diagnosis was established at the age of 13, and strictures had complicated the disease. He had also undergone surgical interventions three times.

In the *CARD15* mutation screening study (study II), all five CD patients with novel *CARD15* mutations had a complicated form of ileal or ileocolonic disease. In addition, R38M and W907R mutation carriers had short bowel syndrome as a complication of several bowel resections. It should also be noted that four of these five patients carried at least one additional *CARD15* mutation.

Since the number of mutation carriers was higher in study II than in study I, genotype-phenotype associations were reanalysed for possible new correlations. A variant was considered a mutation if it resulted in a truncated protein or changed an amino acid that was conserved between species or if the present or previous functional studies had demonstrated impaired function for this protein. Reanalysis did not change the previous results concerning the association with ileal involvement (p=0.03). The complicated form of the disease was associated with double mutation carriers (p=0.045), further strengthening the previous results. Based on the fact that the 1007*fs* mutation was the only variant significantly associated with CD, it was important to study how this mutation affects phenotypic associations. Removal of the 1007*fs* positive patients retained the negative association with pure colonic disease (p=0.011) but the association with ileal location was no longer significant (p=0.11). However, the complicated form of the disease was still associated with mutation carriers as compared to non-carriers (p=0.036).

2. FUNCTIONAL STUDIES OF THE *CARD15* GENE (II)

In order to characterise the functional consequences of the four novel mutations R38M, W355X, P727L, and R1019X in the *CARD15* gene *in vitro*, mononuclear cells of patients were cultured with and without MDP, a ligand for NOD2, and production of interleukin 8 (IL-8) was determined. The fifth novel mutation W907R was not studied because the patient had uraemia. In addition, six wild type CD patients, another six 1007*fs* heterozygous patients, one compound heterozygote for the R702W and 1007*fs* mutation, and two homozygotes for the 1007*fs* mutation were included in the functional studies. Six individuals, negative for the three *CARD15* mutations, from the laboratory staff were used as healthy controls in this functional study. The immune inflammatory status of the individuals was also determined: no significant difference in inflammation status between patient and control groups was observed (study II, Table 2). In addition, no significant difference was observed in lymphocyte and monocyte counts or the proportion of CD14-positive cells between patients and controls.

The results of IL-8 responses showed that three groups, i.e. wild type patients, 1007*fs* heterozygous patients, and healthy controls as well as a compound heterozygote, increased

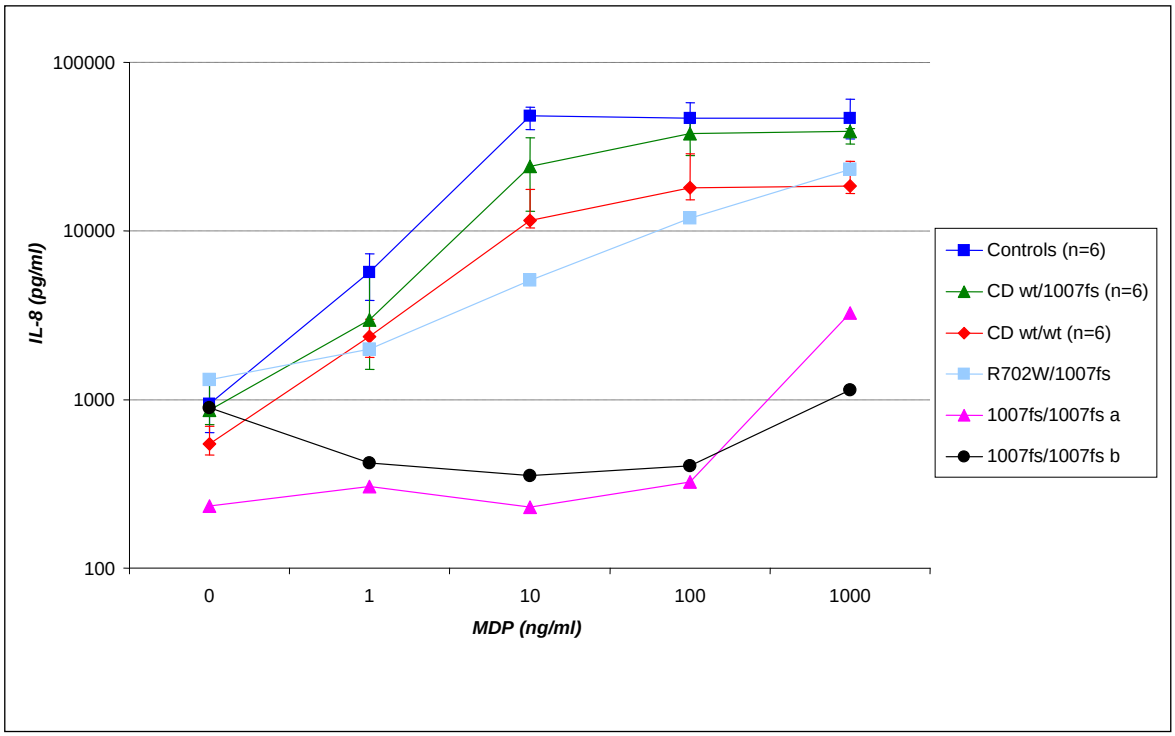

Figure 3. Results of functional studies of known *CARD15* mutations are shown as interleukin 8 responses of peripheral blood mononuclear cells stimulated by muramyl dipeptide (MDP). Data show median and range (bars) of six subjects and the symbols without bars indicate individual patients.

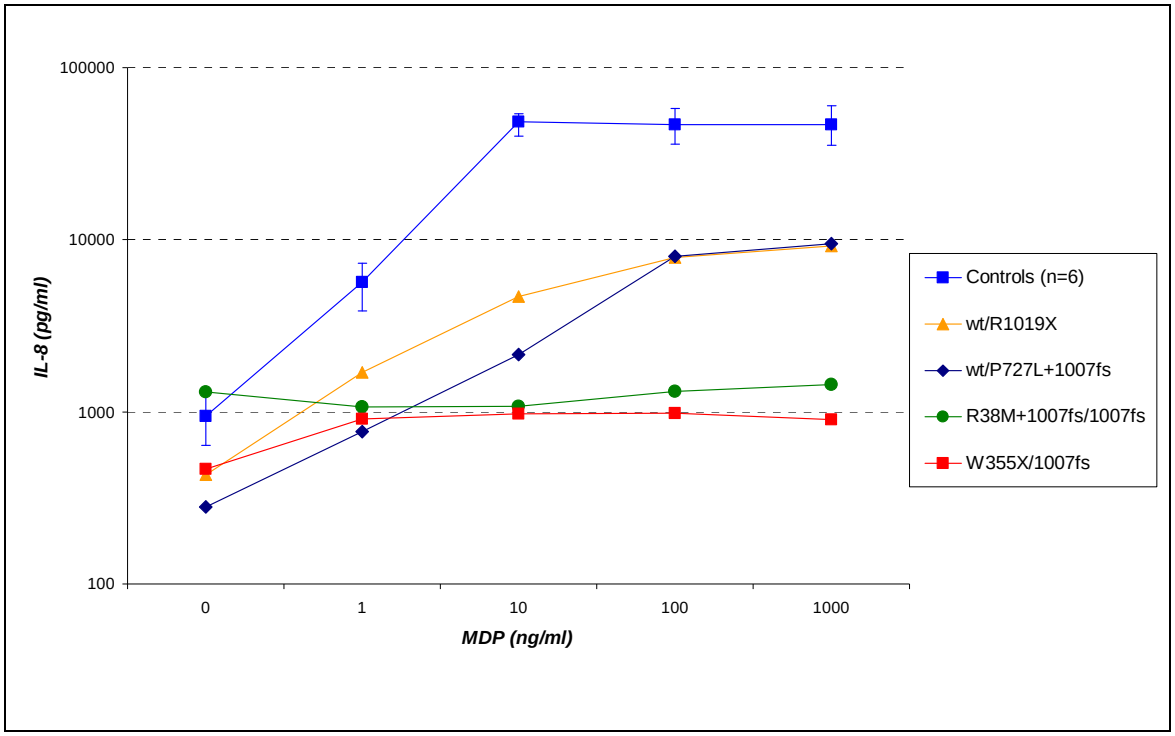

Figure 4. Results of functional studies of novel *CARD15* mutations are shown as interleukin 8 responses of peripheral blood mononuclear cells stimulated by muramyl dipeptide (MDP). The healthy control curve is shown again to permit orientation. All novel *CARD15* mutation curves represent individual patients.

IL-8 production along with MDP concentration in a comparable manner (Figure 3). The corresponding IL-8 levels of each individual are also represented as area under a curve (AUC) using the trapezoidal rule (Figure 5), with no significant difference in AUC values between these three groups. On the contrary, the two 1007*fs* homozygotes had consistently low IL-8 levels (Figure 3), and the respective % AUC values (patient AUC value as a percentage of the lowest control AUC value) were decreased to 12.1% and 5.4% (Figure 5). The range of % AUC values of the 1007*fs* heterozygotes was over 100%, indicating that 1007*fs* heterozygosity was not sufficient to reduce IL-8 production.

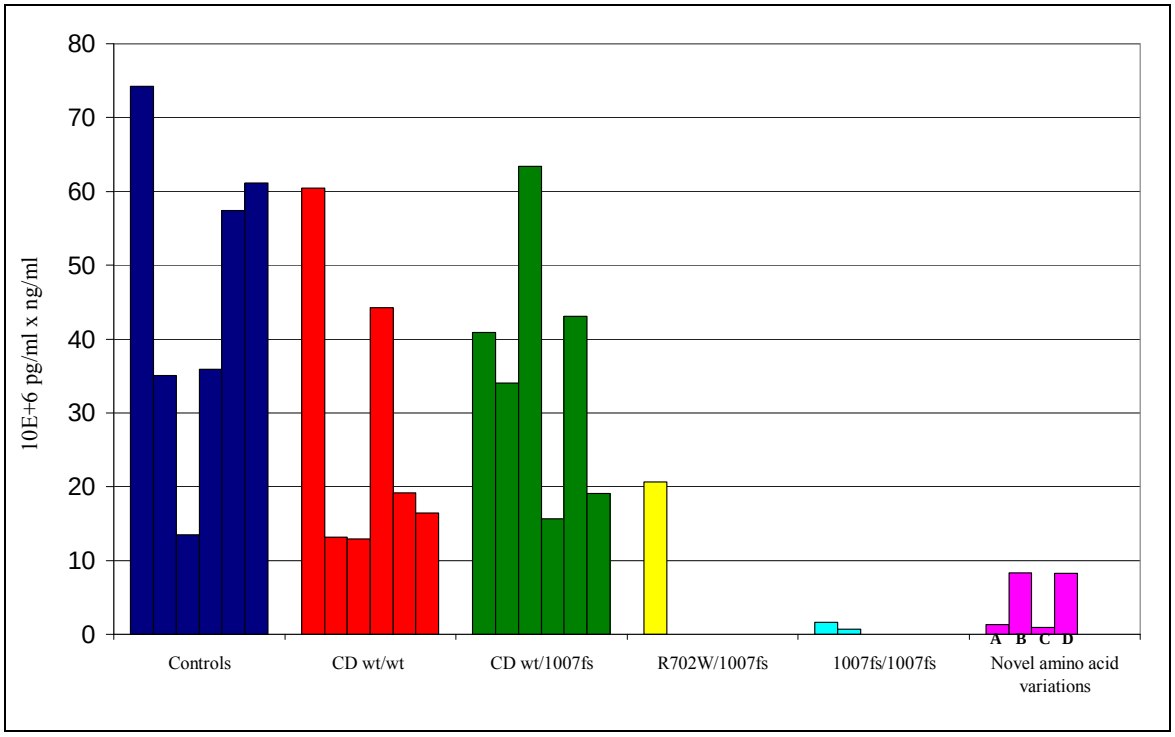

Figure 5. Area under a curve (AUC) values of functional studies. AUC value stands for the serial measurements of IL-8 levels (pg/ml) in response to different muramyl dipeptide (MDP) concentrations (0-1000 ng/ml). Each column symbolizes a single individual. Novel amino acid variations are; A denotes R38M+1007*fs*/1007*fs*, B wt/P727L+1007*fs*, C W355X/1007*fs*, and D wt/R1019X.

Of novel mutations, the patient carrying the W355X variant in conjunction with the 1007*fs* mutation had low IL-8 responses similar to two 1007*fs* homozygotes (Figure 4). This indicates that the two mutations are on different chromosomes and both of these mutations contribute to impaired IL-8 response. The % AUC value of this patient was only 7.0% (Figure 5). The patient with the R38M mutation also had low IL-8 responses (Figure 4) with a 10.1% AUC value (Figure 5) but a firm conclusion about the effect of this mutation can not be made because he was also homozygous for the 1007*fs* mutation. Finally, the P727L and R1019X mutations were also associated with depressed IL-8 production (Figure 4) with % AUC values of 61.4 and 61.9, respectively (Figure 5). It is also of note that the P727L patient was carrying the 1007*fs* mutation on the same allele.

3. GENETIC VARIATIONS IN CANDIDATE GENES AND CORRELATION TO PHENOTYPE

3.1 Screening of the *TRAF6* gene (IV)

Since *TRAF6* presented as a potential IBD candidate gene based on both functional data and the genomic location of the gene in the chromosome 11 IBD linkage region (Paavola-Sakki *et al.* 2003), 95 unrelated IBD patients were screened for mutations in this gene by dHPLC. Only one intronic variation was found, a single nucleotide insertion/deletion polymorphism containing either eight or seven thymine bases (8T/7T), respectively. The location of this variation was eight bases from the intron 3/exon 4 boundary, maintaining the possibility that it affects mRNA splicing. 290 additional CD patients and 416 UC patients were then genotyped for this variation, along with 320 blood donors to obtain a background frequency in the population. Allele and genotype frequencies between different groups were not significantly different. In addition, several clinical characteristics of the patients were analysed because this polymorphism could theoretically affect a sub-phenotype of the disease without affecting the risk of getting the disease itself. Clinical characteristics analysed included the same parameters as in study I. No significant relationship between the polymorphism and IBD phenotypes were found.

3.2 The rare HLA-DRB1*0103 allele (V)

Previous studies have shown strong association of the rare HLA-DRB1*0103 allele with UC, and later also with colonic CD (Ahmad *et al.* 2006). In the present study, 15 UC (3.3%, n=459) and three CD (1.3%, n=240) patients carried the HLA-DRB1*0103 allele in a heterozygous form. In contrast, none of the 190 blood donors carried this allele. The allele frequencies were statistically different between UC and controls (p=0.008) as well as between IBD and controls (p=0.019), but not between CD patients and controls (p=0.26) (study V, Table 3). HLA-DRB1*0103 positive UC patients tended to more often have extensive colitis (67% vs. 61%), and they also needed colectomy more often (47% vs. 29%), but the disease duration was also longer (median years 13.8 vs. 9.7) when compared to patients without this rare allele. These phenotypic differences, however, were not statistically different. All three HLA-DRB1*0103 positive CD patients had a colonic location of the disorder.

3.3 Eight SNPs spanning the *IL23R* gene (V)

Recently, a genome-wide association study reported a highly significant association between CD and *IL23R* (Duerr *et al.* 2006). In the present study, a coding variant R381Q

and seven additional noncoding SNPs in the *IL23R* gene and the intergenic region between the *IL23R* and *IL12RB2* genes were studied in 699 IBD patients and 292 healthy blood donors. Two additional markers rs7517847 and rs1495965 from the original study were excluded due to technical problems. Five SNPs, including the coding variant, were significantly associated with CD (n=240) (study V, Table 1). The strongest association was observed with marker rs2201841 (p=0.002), where the frequency of the C allele was higher in CD patients than in healthy blood donors (37.2% vs. 28.8%, respectively). The other two disease-associated markers rs10889677 and rs1004819, which were in strong LD with rs2201841, had p values of 0.003 and 0.005, respectively (study V, Table 1). The coding variant, rs11209026, was only marginally associated with CD (p=0.045) and the fifth disease-associated marker rs11465804 had a p value of 0.016. After permutation testing the three strongest markers still showed significant association. The results also showed significant association of three markers rs10889677, rs2201841, and rs1004819 with IBD (p=0.026, p=0.029, and p=0.037, respectively) (study V, Table 1).

Haplotype analysis showed nine distinct haplotypes, two of them associating with CD (study V, Table 2). The most common haplotype (TTCTGCAA) was observed to be a risk haplotype with a frequency of 28.8% in CD patients and 23.2% in blood donors (p=0.040). The rare haplotype (CCTGATCG) tagged with the coding variant rs11209026-A allele, which has been shown to confer strong protection against CD, had frequencies of 0.4% in patients and 2.5% in controls (p=0.008).

Genotype-phenotype correlations showed that three *IL23R* markers (rs2201841, rs10889677, and rs1004819) were also associated with longer disease duration among CD patients. Duration with rs2201841 was 5.7 years in wild types, 7.0 in heterozygotes, and 8.1 in homozygotes (p=0.011). The corresponding years with rs10889677 were 5.8, 6.9, and 11.2 (p=0.013) and with rs1004819 5.8, 7.0, and 12.1, respectively (p=0.039). An association between CD and the stricturing phenotype was also observed with the markers rs10889677 and rs2201841. Thus, CD patients with the minor alleles A of rs10889677 and C of rs2201841 more often had stricturing behaviour than patients without these alleles (p=0.010 and p=0.017, respectively).

3.4 Three SNPs within the *TNFRSF1A* gene (V)

Another positional and functional candidate gene *TNFRSF1A* on chromosome 12p13 was analysed by genotyping previously reported polymorphisms: a 5'UTR -609G→T, a coding 36A→G (P12P), and an intronic IVS6+10A→G variant. The allele and genotype frequencies of these three variants did not differ between patient and control groups (study

V, Table 3). However, when the familial (n=114) and sporadic (n=342) UC patients were analysed separately, an association (p=0.03) between familial UC and the rare coding variant 36G was detected. The extension of healthy control material from 190 to 312 individuals further strengthened this association (p=0.007). The association of the other two polymorphisms with familial UC patients, however, did not reach statistical significance.

In genotype-phenotype analysis, the genotype frequencies of the functional variant A36G also differed significantly (p=0.001) between sporadic and familial UC. Accordingly, the AA (wild type) genotype was more frequent in sporadic cases (31.6% vs. 20.2%) whereas the GG (homozygote) genotype was more common in familial cases (32.5% vs. 17.3%). The intronic variant IVS6+10A→G was also associated with familial UC (p=0.042) with AA (wild type) frequencies of 27.2% in familial and 36.0% in sporadic UC. The GG (homozygote) genotype was more common in familial cases (25.4% vs. 15.8%). Comparison of other phenotypic characteristics showed that CD patients carrying the rare coding 36G allele as well as the intronic allele IVS6+10G more often had ileocolonic location than did patients without these two variants (p=0.021 and p=0.028, respectively).

3.5 SNPs in genes *ATG16L1*, *DLG5*, *ABCB1*, *TLR4*, and the chromosome 5 risk haplotype containing *SLC22A4* and *SLC22A5* (V)

A selection of SNPs in the *ATG16L1*, *DLG5*, *ABCB1/MDR1*, *TLR4*, and chromosome risk haplotype containing genes *SLC22A4* and *SLC22A5* were studied for IBD susceptibility. One or two SNPs per candidate gene were studied in a cohort of 699 IBD patients and 190 healthy blood donors. None of these SNPs showed any significant association in the case-control analysis (study V, Table 3). Likewise, carriage of the SLC22A4/5 TC risk haplotype, which has been shown to associate with CD (Peltekova *et al.* 2004), did not differ between patient and control groups.

Genotype-phenotype comparisons showed that the *TLR4* gene polymorphism 299G carriers (72.7%) had more often extensive UC than did D299 carriers (59.6%) (p=0.042).

DISCUSSION

Crohn's disease and ulcerative colitis, collectively known as IBD, are mostly diseases of the twentieth century. Even though the causes of these two diseases are still incompletely understood, the current model is that the intestinal flora drives an aberrant intestinal immune response and inflammation in the genetically susceptible host (Sands 2007). Interest in the genetic of IBD has grown progressively since the familial occurrence of IBD was brought to our attention in the 1960s (Ahmad *et al.* 2001). Molecular genetic studies of IBD started as candidate gene studies already in the 1980s (Orchard *et al.* 2000) but not until a decade later when linkage studies became feasible was real progress in the genetics of IBD achieved. As a result of linkage studies the first CD susceptibility gene, *CARD15*, was found in 2001. The latest progress in molecular genetics has been the advent of GWA studies which have generated enormous data particularly for IBD in a short period of time.

1. GENETIC VARIATIONS IN THE *CARD15* GENE

Three common *CARD15* mutations have been shown to associate with CD in Caucasians. Unlike in previous studies, only 15.5% of Finnish CD patients carried any of the three common mutations. This figure was significantly lower than the corresponding estimates for populations from North America or central and southern Europe. However, the present results are similar to those reported from other Scandinavian countries and Scotland (Arnott *et al.* 2004; Vind *et al.* 2005; Torkvist *et al.* 2006; Ernst *et al.* 2007), which is compatible with our roots (Norio 2003). The present data further confirm the association between the 1007*fs* mutation and CD but there were no significant differences in the allele frequencies of the R702W and G908R mutations between CD patients and the background population. In Sweden and Denmark, two of three common mutations were independently associated with CD (Vind *et al.* 2005; Torkvist *et al.* 2006). In Norway, the R702W and G908R variants had the same allele frequencies among cases and controls, while the 1007*fs* variant had higher mutation frequency in patients but the difference was not statistically significant (Medici *et al.* 2006). In conclusion, the present study on Finnish CD patients emphasizes the importance of the 1007*fs* mutation as a risk allele conferring susceptibility to CD.

In the present study, the 1007*fs* mutation frequency was higher in familial than sporadic patients. It is also of note that two familial probands out of 46 patients and one patient among 198 sporadic patients were homozygous for the 1007*fs* mutation. Higher frequencies of the common *CARD15* mutations among familial patients has also been

reported for other populations (Cuthbert *et al.* 2002; Zhou *et al.* 2002; Oostenbrug *et al.* 2006b) but some studies have been unable to confirm this (Newman *et al.* 2004; Annese *et al.* 2005a; van der Linde *et al.* 2007).

In the present *CARD15* screening study, 30 different sequence variants were detected, half the number of variants found in a large European study (Lesage *et al.* 2002). There was no evidence for a putative Finnish founder or major mutation, but instead five novel mutations (R38M, W355X, P727L, W907R, and R1019X) with apparent pathophysiological roles in CD were detected. The 1007*fs* variant remained the only mutation that associated significantly with CD. All novel mutations except the W907R variant were tested for functional consequences *in vitro*.

Two novel variants (W355X and R1019X) were nonsense mutations like the common 1007*fs* mutation. Functional studies on the W355X mutation showed that this mutation has an inhibitory effect on IL-8 production similar to the 1007*fs* mutation. It was impossible to judge whether the W355X and 1007*fs* mutations were on the same chromosome since other family members were unavailable. Nevertheless, the poor IL-8 production similar to 1007*fs* homozygotes strongly suggests that these two mutations are on different alleles. R1019X is very similar to the 1007*fs* mutation in that they both reside in the LRR domain. The patient carrying the R1019X mutation showed lower IL-8 production than did the 1007*fs* heterozygous patients, indicating that the R1019X mutation could be the more severe of these two mutations. It is interesting that van Heel and co-workers found one CD patient carrying the R1019G mutation (van Heel *et al.* 2006). This mutation, however, was not shown to have impaired IL-8 production.

The other two novel variants R38M and P727L were missense mutations. Since the R38M mutation occurred in a 1007*fs* homozygous patient, firm conclusions about its functional consequences can not be made. The P727L mutation also co-occurred with the 1007*fs* mutation. However, high degrees of cross-species conservation of the respective wild type amino acids at these positions and the chemical nature of the changes involved suggest the pathogenic significance of these variants. Substitution of arginine to methionine at position 38 replaces a hydrophilic and polar amino acid to a neutral and nonpolar one. Replacement of proline by leucine at position 727 may radically change the overall structure of the encoded protein. Furthermore, functional studies showed that the patient with mutations P727L and 1007*fs* on the same chromosome had, unlike the pure 1007*fs* heterozygotes, strongly depressed IL-8 production. This was most likely due to presence of two mutations on the same chromosome.

The fifth novel variant W907R most likely has impaired function based on the previous observation made for an analogous mutation W907L. This particular mutation has shown to be a loss of function mutation as W907 is involved in MDP binding and/or protein-protein interaction required for MDP recognition (Tanabe *et al.* 2004). In addition, W907 is a conserved amino acid based on cross-species comparisons.

There are some functional data available for the previously reported variants N289S, T294S, R703C, M863V, and V955I (Lesage *et al.* 2002; Miceli-Richard *et al.* 2002; Sugimura *et al.* 2003a; Sun *et al.* 2003; Lakatos *et al.* 2004; Tukel *et al.* 2004; Lakatos *et al.* 2005; King *et al.* 2006; Schnitzler *et al.* 2006; van Heel *et al.* 2006), identified also in the present study, as well as for the common mutations R702W and G908R. Human embryonic kidney (HEK) 293 cells transfected with R702W and G908R were characterised by at least a two fold reduction in both basal NF-κB activity and PGN-induced response, indicating a constitutive defect in *CARD15* function (Bonen *et al.* 2003; Chamaillard *et al.* 2003). The rare variant N289S which was present in one CD patient has been shown to have similar functional characteristics (Chamaillard *et al.* 2003). On the contrary, the responses of T294S, R703C, M863V, and V955I variants to PGN were similar to the wild type allele (Chamaillard *et al.* 2003). Additional data supporting the normal function of the R703C variant comes from mononuclear cell study which showed no profound loss of MDP-sensing with this variant (van Heel *et al.* 2006). Some studies, however, have suggested that the R703C variant is a potential disease-susceptibility allele based on sequence alignments, use of different prediction programs, and case-control analysis (Lesage *et al.* 2002; Sun *et al.* 2003; Tukel *et al.* 2004; Lakatos *et al.* 2005; King *et al.* 2006).

The previously described variants A755V (Lesage *et al.* 2002; Miceli-Richard *et al.* 2002; Lakatos *et al.* 2005; Schnitzler *et al.* 2006; van Heel *et al.* 2006) and R791Q (Miceli-Richard *et al.* 2002; Sugimura *et al.* 2003a; Lakatos *et al.* 2005) are conserved across species. Functional studies on the A755V variant co-occurring with the 1007*fs* mutation have shown a profound impairment of MDP responses (van Heel *et al.* 2006). In the present study, the allele frequencies of the A755V variant were higher in CD patients than in the background population, but this difference was not statistically significant. A higher 755V frequency in CD patients than controls has also been reported in another study (Lesage *et al.* 2002), but the number of mutated chromosomes was also relatively low. For the R791Q variant found in one CD patient no functional data are available at the moment.

The present data from structural analysis of the *CARD15* gene support the idea that rare *CARD15* mutations account only a small fraction of the susceptibility to CD. It is still

possible, however, that there are regulatory mutations in the 5'UTR or intronic areas of the gene and they contribute to the genetic susceptibility to CD.

The present study, using criteria based on the biochemical nature of the amino acid changes, allele frequencies in case-control analyses, and functional data available from the present and previous studies, suggests that 3% of Finnish CD patients carried two disease-susceptibility mutations while 16% carried one such mutation. These figures are much lower than those reported in the European collaboratory study where 17% of CD patients carried two mutations and 50% carried one mutation (Lesage *et al.* 2002). These results, combined with similar findings in Scotland (Arnott *et al.* 2004) and Scandinavia (Hampe *et al.* 2002b; Vind *et al.* 2005; Medici *et al.* 2006; Torkvist *et al.* 2006; Ernst *et al.* 2007), suggest the existence of other contributing susceptibility loci in CD.

1.1 *CARD15* mutation frequency in the background population

The present study on *CARD15* mutation frequency in healthy controls is the largest of its type to date. Data were obtained from 15 IBD research groups on three continents. It should be emphasized that these population samples were recruited by different methods, and most of them were not well characterised. The major bias that might affect the conclusions is the possibility of including actual CD patients in the background population. Furthermore, CD may not have developed yet in the youngest population controls screened.

Results from the present study and the literature show that in Caucasians, large differences are observed in the frequency of the three common mutations among apparently healthy individuals. In Europe, the *CARD15* mutation frequency is relatively low in northern parts including Scotland and Scandinavia (Medici *et al.* 2006; Torkvist *et al.* 2006) and the highest in central European countries Ireland (Bairead *et al.* 2003), England, Belgium, France, Croatia (Cukovic-Cavka *et al.* 2006), Germany, and Hungary (Lakatos *et al.* 2005). In southern Europe, including Spain (Mendoza *et al.* 2003; Nunez *et al.* 2004), Portugal (Ferreira *et al.* 2005), and Italy, the mutation frequency is intermediate with the exception of Greece where it is compatible with central Europe (Gazouli *et al.* 2005b). These data are in accordance with more general observations of north-south gradients for genetic polymorphisms through Europe that are similar to the routes taken by Neolithic farmers from the Levant 10 000 years ago (Barbujani and Bertorelle 2001).

The *CARD15* mutation frequency, however, is very low in Turkey (Uyar *et al.* 2004) and Israeli Arabs (Karban *et al.* 2005), whereas in Tunisia and Sephardic Jewish populations

from North Africa the *CARD15* mutation frequency is somewhat higher (Tukel *et al.* 2004; Zouiten-Mekki *et al.* 2005). In North America, the mutation frequencies are similar to those observed in central Europe except for Quebec, which has a lower frequency. Finally, in the Pacific, the frequency is similar to southern Europe (Gearry *et al.* 2006). Altogether, these data demonstrate that a large geographic heterogeneity exists in Caucasians and is most likely due to population history, genetic drift, admixture, and migrations.

The large differences observed in *CARD15* mutation frequency between populations could indicate that it reflects the incidence of CD. Comparison of incidences and mutation prevalence could not, however, show a clear relationship. For example, in Finland CD incidence is relatively high (5.6/100 000) but the mutation frequency is low, while in Italy the situation is the opposite, having high mutation frequency with relatively low incidence (2.1/100 000). Lack of a clear relationship between mutation frequency in the background population and CD incidence indicate that *CARD15* is only one of many different factors contributing to disease development and is not alone sufficient to influence disease incidence. Results from double mutation carriers indicate that the majority of mutation carriers are healthy and disease penetrance is limited, as expected for complex genetic diseases. This conclusion is also in accordance with incomplete disease concordance in monozygotic twins carrying the *CARD15* mutations (Jess *et al.* 2005).

1.2 Genotype-phenotype correlations of *CARD15* mutations

Previous studies have consistently shown association between the *CARD15* mutations and an ileal location of CD. In the present study, the same association was confirmed in the Finnish CD material. It was also seen that the association was dependent on the occurrence of the 1007*fs* mutation. Stricturing and/or penetrating phenotypes have also been reported to associate with the common *CARD15* mutations (Abreu *et al.* 2002; Hampe *et al.* 2002b; Lesage *et al.* 2002; Brant *et al.* 2003b; Economou *et al.* 2004; Heresbach *et al.* 2004; Oostenbrug *et al.* 2006b; van der Linde *et al.* 2007). This association was also observed in the present study: patients with two mutations more frequently had complicated phenotype than patients without or with one mutation.

In some studies, carrier status for two mutations has shown to associate with an earlier age at onset of CD (Lesage *et al.* 2002; Brant *et al.* 2003b; Newman *et al.* 2004; Annese *et al.* 2005a; Oostenbrug *et al.* 2006b). In the present CD cohort, two sibling pairs and one sporadic patient, who were homozygous for the 1007*fs* mutation, also had earlier ages at onset. In addition, these patients had more severe disease as indicated by occurrence of a

stricturing phenotype. This is in accordance with the study of Lesage and colleagues (Lesage *et al.* 2002), who showed that patients with two mutations had stricturing phenotype more often than patients without mutation.

2. GENETIC VARIATION IN OTHER IBD CANDIDATE GENES

2.1 Positive association with *IL23R*, *TNFRSF1A*, and HLA-DRB1*0103 allele variants

Since the original report concerning the *IL23R* gene with IBD and especially with CD susceptibility (Duerr *et al.* 2006), three other GWA studies have confirmed this finding (Consortium 2007; Libioulle *et al.* 2007; Raelson *et al.* 2007). Many replication studies have also reported association to both CD and UC (Buning *et al.* 2007; Cummings *et al.* 2007a; Glas *et al.* 2007; Oliver *et al.* 2007; Roberts *et al.* 2007; Tremelling *et al.* 2007; Weersma *et al.* 2008) while some have concentrated on CD only (Borgiani *et al.* 2007). In the present study, the involvement of the *IL23R* gene only with CD susceptibility was confirmed. In comparison to previous studies, a slightly weaker association to CD was obtained, possibly due to the facts that the present CD cohort was somewhat smaller and unselected instead of only ileal CD cases were examined.

The protective coding variant (R381Q) of the *IL23R* gene has shown the strongest association in most studies although the minor allele frequency is very low in all populations. The frequency of this protective variant was even lower in the present IBD cohort, and only six CD patients were heterozygous for the minor allele A. These patients shared no common clinical characteristics. Duerr and colleagues found an *IL23R* haplotype protective against CD (Duerr *et al.* 2006). It is also of note that the protective haplotype in the Finnish CD patients (C***C***TGATCG) differed by only one nucleotide from the haplotype in North American Caucasian CD patients (C***G***TGATCG). In addition, a significant association of two *IL23R* SNPs with stricturing behaviour of CD was demonstrated, and three SNPs were also associated with longer disease duration, both of which have only been reported in the present study.

In our previous genome-wide linkage scan, the highest two-point NPL score of 2.15 for IBD and the second highest NPL score of 2.00 for UC were observed on chromosome 12p13 (Paavola-Sakki *et al.* 2003). This chromosomal region also harbours the *TNFRSF1A* gene. When UC families were stratified according to their age at onset (≤24 years at diagnosis), the two-point NPL score reached 2.72. The present study shows that the *TNFRSF1A* gene variants A36G and IVS6+10A→G were significantly associated with the familial, but not sporadic, form of UC. Evidence was also obtained suggesting that the

same alleles were markers of ileocolonic involvement in CD. Other investigators have previously reported association between the *TNFRSF1A* A36G variant and CD as well as a negative association with stricturing phenotype (Waschke *et al.* 2005). Association of the same variant with the pancolitis phenotype of UC has been observed in the Belgian population (Pierik *et al.* 2004). No association between the A36G variant and IBD in Japanese population has been observed (Sashio *et al.* 2002).

The HLA-DRB1*0103 allele is very rare with less than 2% frequency in Caucasian populations but nevertheless it is the most replicated HLA class II allele in UC susceptibility (Ahmad *et al.* 2006). The association between the HLA-DRB1*0103 allele and UC was identified for the first time in 1996 (Satsangi *et al.* 1996b), and with CD a few years later (Trachtenberg *et al.* 2000). The present study confirms the association of this rare allele with UC among Finnish IBD patients, and the trend was similar for CD. The most consistent phenotypic findings among HLA-DRB1*0103 positive UC patients have been extensive colitis and severe disease as defined by the need for colectomy (Satsangi *et al.* 1996b; Roussomoustakaki *et al.* 1997; Bouma *et al.* 1999; Ahmad *et al.* 2003; Yamamoto-Furusho *et al.* 2003). In HLA-DRB1*0103 positive CD patients, a colonic location has been the most consistent finding (Silverberg *et al.* 2003; Fernandez *et al.* 2004; Newman *et al.* 2004). Extensive disease and need for colectomy as well as longer disease duration were more common in the present series of HLA positive UC patients but probably because of the overall rarity of this allele these phenotypic parameters were not statistically significant. In addition, all three CD patients carrying the HLA-DRB1*0103 allele had colonic involvement. These data strongly link the HLA-DRB1*0103 allele to inherited susceptibility of colonic inflammation, regardless of the exact subtype of IBD.

2.2 No association with *TRAF6*, *ATG16L1*, *DLG5*, *ABCB1*, *TLR4*, *SLC22A4*, *SLC22A5*, or the chromosome 5 haplotype

Only one intronic variant was detected upon *TRAF6* gene screening. This variation, containing either seven or eight thymine (T) bases close to the intron 3/exon 4 boundary, was of potential interest because variable lengths of poly(T) tracts have been shown to associate with other conditions. For example, variable lengths of poly(T) tracts present close to the intron 8 splice branch/acceptor site result in alternative splicing of the human cystic fibrosis transmembrane conductance regulator (*CFTR*) mRNA (Chu *et al.* 1991; Chu *et al.* 1993; Chillon *et al.* 1995). In addition, mutations at 11 poly(T) repeat in intron 4 of the DNA repair protein *MRE11* gene have been reported to cause aberrant splicing of mRNA and the generation of truncated proteins (Giannini *et al.* 2002). The opposite has been seen in the human interferon gamma (*IFNG*) gene where the different lengths of

poly(T) close to the splice acceptor site of intron 1 did not alter gene expression levels (Bream *et al.* 2000). Since the present study failed to show any evidence of disease association with the *TRAF6* poly(T) tract, there was no point to carry on with mRNA splicing experiments.

This study was unable to confirm previous findings on the associations of *ATG16L1*, *DLG5*, *ABCB1*, or *TLR4* variants, or the chromosome 5 risk haplotype including *SLC22A4* and *SLC22A5*, with IBD. It is, however, possible that the number of patients studied was too small to detect an association, even if it is present in Finns. A phenotypic analysis showed some evidence for association between the *TLR4* D299G polymorphism and extensive UC, but due to the lack of association in case-control analysis the significance of this finding remains obscure.

CONCLUDING REMARKS AND FUTURE PROSPECTS

IBD is a common disease of industrialised and developed countries, and the incidence has been growing since the 1950s. Management of IBD is often difficult, with recurrent symptoms and significant morbidity, and therefore treatment and hospitalisation are frequently associated with high cost and significant use of human resources.

One of the main goals was to assess the role of the *CARD15* gene in a Finnish CD cohort. Three common *CARD15* mutations R702W, G908R, and 1007*fs* associate independently with CD susceptibility, and the present data showed that 16% of CD patients carried one of the three common mutations. Only the 1007*fs* mutation associated significantly with CD. As a result of *CARD15* screening, five novel amino acid changes (R38M, W355X, P727L, W907R, and R1019X) were found. Novel variants are most likely disease-causing mutations on the basis of *in vitro* functional studies, cross-species comparisons, and the nature of the amino acid substitutions. At the moment, *CARD15* genotyping does not help much in the establishment of diagnosis or selection of treatment, and thus it is not routinely recommended to screen CD patients for *CARD15* mutations.

Allele frequencies of the three common *CARD15* mutations showed remarkable differences in apparently healthy individuals across three continents. The proportion of double mutant carriers showed that CD penetrance is limited, as expected for complex disease. No clear relationship was observed between *CARD15* mutation frequncy and disease incidence in different countries, indicating that there must be other factors involved in CD susceptibility.

Investigation of some of the recently described candidate genes also revealed an association of *IL23R* and the HLA-DRB1*0103 allele with CD and UC susceptibility, respectively. In addition, an association between the *TNFRSF1A* gene and familial UC was identified.

This is the first molecular genetic study concerning IBD in Finland. During the last decade there has been remarkable progress in the field of IBD genetics, and it is very likely that additional susceptibility genes will soon be found. High density mapping of potential loci identified from the Finnish genome scan and the conducting of a GWA study on Finnish UC patients might help to identify novel susceptibility genes. Identification of new genes may increase our understanding of the aetiology of IBD and could eventually improve diagnosis and assist in tailoring treatments for the disease.

ACKNOWLEDGEMENTS

The current study was carried out at the Research Program for Molecular Medicine, Biomedicum Helsinki and at the Department of Medicine, University of Helsinki during the years 2002-2008. The research project was part of the Centre of Excellence in Complex Disease Genetics (years 2006-2011) of the Academy of Finland. I wish to express my gratitude to professors Reijo Tilvis and Olavi Ylikorkala, the former and present heads of the Institute of Clinical Medicine, and professors Kimmo Kontula and Vuokko Kinnula, the former and present heads of the Department of Medicine, for providing excellent research facilities.

This study was financially supported by the Special State Share of the Helsinki University Central Hospital, the Research Funds of the University of Helsinki, and the Clinical Chemistry Research Foundation.

I am deeply indebted to my supervisors Dr Paulina Paavola-Sakki and professor Kimmo Kontula for guiding me through this research project. Paulina, you have been supportive when times have been rough, and I have enjoyed your company at our mutual IBD Consortium meetings. Kimmo, you are always so chronicly interested in science and, in addition, you are an incredible scientific writer which never stops amazing me.

I am very grateful to docents Irma Järvelä and Kaija-Leena Kolho for carefully reviewing my thesis and for the most valuable comments and constructive criticism. Jodie Painter is acknowledged for thoroughly revising the language of my thesis.

I am indebted to our clinicians docents Martti Färkkilä, Leena Halme, and Tiina Heliö as well as Dr Ulla Turunen whose role in my thesis has been invaluable. This thesis would have not been possible without your effort collecting the patient material. Leena and Ulla are also acknowledged for reviewing the clinical part of my thesis. I am very grateful for the patients and families who have participated in the present study.

I wish to thank all my collaborators. Docent Heikki Repo and Eine Virolainen, at the Department of Bacteriology and Immunology, University of Helsinki, are acknowledged for invaluable collaboration. You also made me feel very welcome to your lab. My very warm thanks go to other collaborators docent Päivi Saavalainen and Elisabet Einarsdottir at the Research Program for Molecular Medicine as well.

Raija Selivuo is acknowledged for kind help with numerous practical matters.

I would like to thank Saara Nyqvist, Tuula Soppela, and Susanna Tverin for excellent technical assistance. In addition, I would like to acknowledge Tuula and Susanna for the friendship we share.

I have been honored to have the opportunity to work with many wonderful colleagues and friends. When I started at Kimmo's lab, my closest colleagues were Heidi Fodstad, Päivi Forsblom, and Kaisa Valli-Jaakola. I have spent really good and memorable times with you at work and outside the work. Tuula Hannila-Handelberg, Annukka Marjamaa, Annukka Lahtinen, and Timo Suonsyrjä, I would like to wish all the best for your own thesis projects. This feeling is really something it is worth of doing. Present and former members of the group, Timo Hiltunen, Jukka Lehtonen, Marika Lilja, Maiju Merisalo, Laura Oksanen, Kristian Paavonen, Kirsi Paukku, and Kirsi Piippo are remembered with warmth. Kati Donner, the last but not least of our group member, you have always been there for me with both work and personal matters. I am truly happy we have made such a good friendship in a short period of time.

I wish to thank people from professor Aarno Palotie's lab: Kirsi Alakurtti, Verneri Anttila, Eija Hämäläinen, Mari Kaunisto, Riitta Sallinen, Annika Sarahonka, Päivi Tikka-Kleemola, and docent Maija Wessman. Maija, you are the one who introduced me into this field and I am really grateful for that. I would like to mention Paula Kokko, Merja Lahtinen, Susanna Mehtälä, and Jaana Valkeapää for the pleasant company at coffee breaks.

I would like to thank my friends for endless support during this journey. The deepest gratitude goes to my parents Ulla and Pentti for continuous love and support. You have always trusted in me and let me do my own decisions in life. My sister Johanna has also been invaluable to me. Mikko's parents Pirjo and Esko as well as his sister Paula along with her family have given me memorable moments outside the work, especially on our mutual trips abroad.

Finally, my dear life-companion Mikko, you have been there almost from the very beginning and stayed througout this project. I am really grateful that you gave me all the time I needed at the end of this project. I am glad to have you in my life!

Helsinki, April 2008

REFERENCES

Abbott DW, Wilkins A, Asara JM, and Cantley LC (2004). The Crohn's disease protein, NOD2, requires RIP2 in order to induce ubiquitinylation of a novel site on NEMO. *Curr Biol* 14(24):2217-27.

Abbott DW, Yang Y, Hutti JE, Madhavarapu S, Kelliher MA, and Cantley LC (2007). Coordinated regulation of Toll-like receptor and NOD2 signaling by K63-linked polyubiquitin chains. *Mol Cell Biol* 27(17):6012-25.

Abreu MT, Taylor KD, Lin Y-C, Hang T, Gaiennie J, Landers CJ, Vasiliauskas EA, Kam LY, Rojany M, Papadakis KA, Rotter JI, Targan SR, and Yang H (2002). Mutations in NOD2 are associated with fibrostenosing disease in patients with Crohn's disease. *Gastroenterology* 123(3):679-688.

Abreu MT, and Sparrow MP (2006). Translational research in inflammatory bowel disease. *Mt Sinai J Med* 73(8):1067-73.

Achkar JP, Dassopoulos T, Silverberg MS, Tuvlin JA, Duerr RH, Brant SR, Siminovitch K, Reddy D, Datta LW, Bayless TM, Zhang L, Barmada MM, Rioux JD, Steinhart AH, McLeod RS, Griffiths AM, Cohen Z, Yang H, Bromfield GP, Schumm P, Hanauer SB, Cho JH, and Nicolae DL (2006). Phenotype-stratified genetic linkage study demonstrates that IBD2 is an extensive ulcerative colitis locus. *Am J Gastroenterol* 101(3):572-80.

Aderem A, and Ulevitch RJ (2000). Toll-like receptors in the induction of the innate immune response. *Nature* 406(6797):782-7.

Aggarwal BB (2003). Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol* 3(9):745-756.

Ahmad T, Satsangi J, McGovern D, Bunce M, and Jewell DP (2001). Review article: the genetics of inflammatory bowel disease. *Aliment Pharmacol Ther* 15(6):731-48.

Ahmad T, Armuzzi A, Bunce M, Mulcahy-Hawes K, Marshall SE, Orchard TR, Crawshaw J, Large O, De Silva A, Cook JT, Barnardo M, Cullen S, Welsh KI, and Jewell DP (2002). The molecular classification of the clinical manifestations of Crohn's disease. *Gastroenterology* 122(4):854-866.

Ahmad T, Armuzzi A, Neville M, Bunce M, Ling KL, Welsh KI, Marshall SE, and Jewell DP (2003). The contribution of human leucocyte antigen complex genes to disease phenotype in ulcerative colitis. *Tissue Antigens* 62(6):527-35.

Ahmad T, Marshall SE, and Jewell D (2006). Genetics of inflammatory bowel disease: the role of the HLA complex. *World J Gastroenterol* 12(23):3628-35.

Akira S, and Takeda K (2004). Toll-like receptor signalling. *Nat Rev Immunol* 4(7):499-511.

Akira S, Uematsu S, and Takeuchi O (2006). Pathogen recognition and innate immunity. *Cell* 124(4):783-801.

Amos CI (2007). Successful design and conduct of genome-wide association studies. *Hum Mol Genet* 16 Spec No 2:R220-5.

Annese V, Latiano A, Bovio P, Forabosco P, Piepoli A, Lombardi G, Andreoli A, Astegiano M, Gionchetti P, Riegler G, Sturniolo GC, Clementi M, Rappaport E, Fortina P, Devoto M, Gasparini P, and Andriulli A (1999). Genetic analysis in Italian families with inflammatory bowel disease supports linkage to the IBD1 locus--a GISC study. *Eur J Hum Genet* 7(5):567-73.

Annese V, Lombardi G, Perri F, D'Inca R, Ardizzone S, Riegler G, Giaccari S, Vecchi M, Castiglione F, Gionchetti P, Cocchiara E, Vigneri S, Latiano A, Palmieri O, and Andriulli A (2005a). Variants of CARD15 are associated with an aggressive clinical course of Crohn's disease--an IG-IBD study. *Am J Gastroenterol* 100(1):84-92.

Annese V, Piepoli A, Latiano A, Lombardi G, Napolitano G, Caruso N, Cocchiara E, Accadia L, Perri F, and Andriulli A (2005b). HLA-DRB1 alleles may influence disease phenotype in patients with inflammatory bowel disease: a critical reappraisal with review of the literature. *Dis Colon Rectum* 48(1):57-64; discussion 64-5.

Annese V, Valvano MR, Palmieri O, Latiano A, Bossa F, and Andriulli A (2006). Multidrug resistance 1 gene in inflammatory bowel disease: a meta-analysis. *World J Gastroenterol* 12(23):3636-44.

Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, Frees K, Watt JL, and Schwartz DA (2000). TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 25(2):187-91.

Ardizzone S, Maconi G, Bianchi V, Russo A, Colombo E, Cassinotti A, Penati C, Tenchini ML, and Bianchi Porro G (2007). Multidrug resistance 1 gene polymorphism and susceptibility to inflammatory bowel disease. *Inflamm Bowel Dis* 13(5):516-23.

Armuzzi A, Ahmad T, Ling KL, de Silva A, Cullen S, van Heel D, Orchard TR, Welsh KI, Marshall SE, and Jewell DP (2003). Genotype-phenotype analysis of the Crohn's disease susceptibility haplotype on chromosome 5q31. *Gut* 52(8):1133-9.

Arnott ID, Nimmo ER, Drummond HE, Fennell J, Smith BR, MacKinlay E, Morecroft J, Anderson N, Kelleher D, O'Sullivan M, McManus R, and Satsangi J (2004). NOD2/CARD15, TLR4 and CD14 mutations in Scottish and Irish Crohn's disease patients: evidence for genetic heterogeneity within Europe? *Genes Immun* 5(5):417-25.

Arnott ID, Ho GT, Nimmo ER, and Satsangi J (2005). Toll-like receptor 4 gene in IBD: further evidence for genetic heterogeneity in Europe. *Gut* 54(2):308; author reply 309.

Babusukumar U, Wang T, McGuire E, Broeckel U, and Kugathasan S (2006). Contribution of OCTN variants within the IBD5 locus to pediatric onset Crohn's disease. *Am J Gastroenterol* 101(6):1354-61.

Bairead E, Harmon DL, Curtis AM, Kelly Y, O'Leary C, Gardner M, Leahy DT, Vaughan P, Keegan D, O'Morain C, O'Donoghue D, Shanahan F, Parfrey NA, and Quane KA (2003). Association of NOD2 with Crohn's disease in a homogenous Irish population. *Eur J Hum Genet* 11(3):237-44.

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, and Hakonarson H (2007a). Association of the T300A non-synonymous variant of the ATG16L1 gene with susceptibility to paediatric Crohn's disease. *Gut* 56(8):1171-3.

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, and Hakonarson H (2007b). Association of Variants of the Interleukin-23 Receptor Gene With Susceptibility to Pediatric Crohn's Disease. *Clin Gastroenterol Hepatol* 5(8):972-976.

Bamias G, and Cominelli F (2007). Immunopathogenesis of inflammatory bowel disease: current concepts. *Curr Opin Gastroenterol* 23(4):365-9.

Barbujani G, and Bertorelle G (2001). Genetics and the population history of Europe. *Proc Natl Acad Sci U S A* 98(1):22-5.

Barmada MM, Brant SR, Nicolae DL, Achkar JP, Panhuysen CI, Bayless TM, Cho JH, and Duerr RH (2004). A genome scan in 260 inflammatory bowel disease-affected relative pairs. *Inflamm Bowel Dis* 10(5):513-20.

Barnich N, Aguirre JE, Reinecker H-C, Xavier R, and Podolsky DK (2005). Membrane recruitment of NOD2 in intestinal epithelial cells is essential for nuclear factor-{kappa}B activation in muramyl dipeptide recognition. *J. Cell Biol.* 170(1):21-26.

Baron M (2001). The search for complex disease genes: fault by linkage or fault by association? *Mol Psychiatry* 6(2):143-9.

Barton GM, and Medzhitov R (2003). Toll-like receptor signaling pathways. *Science* 300(5625):1524-5.

Baumgart DC, and Carding SR (2007). Inflammatory bowel disease: cause and immunobiology. *Lancet* 369(9573):1627-40.

Baumgart DC, and Sandborn WJ (2007). Inflammatory bowel disease: clinical aspects and established and evolving therapies. *Lancet* 369(9573):1641-57.

Beg AA, and Baltimore D (1996). An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. *Science* 274(5288):782-4.

Bene J, Magyari L, Talian G, Komlosi K, Gasztonyi B, Tari B, Varkonyi A, Mozsik G, and Melegh B (2006). Prevalence of SLC22A4, SLC22A5 and CARD15 gene mutations in Hungarian pediatric patients with Crohn's disease. *World J Gastroenterol* 12(34):5550-3.

Benoni C, and Nilsson A (1987). Smoking habits in patients with inflammatory bowel disease. A case-control study. *Scand J Gastroenterol* 22(9):1130-6.

Berg DF, Bahadursingh AM, Kaminski DL, and Longo WE (2002). Acute surgical emergencies in inflammatory bowel disease. *Am J Surg* 184(1):45-51.

Bernstein CN, Blanchard JF, Rawsthorne P, and Wajda A (1999). Epidemiology of Crohn's disease and ulcerative colitis in a central Canadian province: a population-based study. *Am J Epidemiol* 149(10):916-24.

Berrebi D, Maudinas R, Hugot JP, Chamaillard M, Chareyre F, De Lagausie P, Yang C, Desreumaux P, Giovannini M, Cezard JP, Zouali H, Emilie D, and Peuchmaur M (2003). Card15 gene overexpression in mononuclear and epithelial cells of the inflamed Crohn's disease colon. *Gut* 52(6):840-6.

Best WR, Becktel JM, Singleton JW, and Kern F, Jr. (1976). Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. *Gastroenterology* 70(3):439-44.

Biank V, Friedrichs F, Babusukumar U, Wang T, Stoll M, Broeckel U, and Kugathasan S (2007). DLG5 R30Q variant is a female-specific protective factor in pediatric onset Crohn's disease. *Am J Gastroenterol* 102(2):391-8.

Blau EB (1985). Familial granulomatous arthritis, iritis, and rash. *J Pediatr* 107(5):689-93.

Bonen DK, Niclolae DL, and Moran T (2002). Racial differences in Nod2 variation: characterization of Nod2 in African-Americans with Crohn's disease. *Gastroenterology* 122(Suppl):A-29.

Bonen DK, and Cho JH (2003). The genetics of inflammatory bowel disease. *Gastroenterology* 124(2):521-36.

Bonen DK, Ogura Y, Nicolae DL, Inohara N, Saab L, Tanabe T, Chen FF, Foster SJ, Duerr RH, Brant SR, Cho JH, and Nunez G (2003). Crohn's disease-associated NOD2 variants share a signaling defect in response to lipopolysaccharide and peptidoglycan. *Gastroenterology* 124(1):140-146.

Borgiani P, Perricone C, Ciccacci C, Romano S, Novelli G, Biancone L, Petruzziello C, and Pallone F (2007). Interleukin-23R Arg381Gln Is Associated With Susceptibility to Crohn's Disease But Not With Phenotype in an Italian Population. *Gastroenterology* 133(3):1049-1051.

Borruel N, Carol M, Casellas F, Antolin M, de Lara F, Espin E, Naval J, Guarner F, and Malagelada JR (2002). Increased mucosal tumour necrosis factor alpha production in Crohn's disease can be downregulated ex vivo by probiotic bacteria. *Gut* 51(5):659-64.

Bouma G, Crusius JBA, Garcia-Gonzalez MA, Meijer BUGA, Hellemans HPR, Hakvoort RJ, Th. Schreuder GM, Kostense PJ, Meuwissen SGM, and Pena AS (1999). Genetic markers in clinically well defined patients with ulcerative colitis (UC). *Clinical & Experimental Immunology* 115(2):294-300.

Bouma G, and Strober W (2003). The immunological and genetic basis of inflammatory bowel disease. *Nat Rev Immunol* 3(7):521-33.

Bourhis LL, Benko S, and Girardin SE (2007). Nod1 and Nod2 in innate immunity and human inflammatory disorders. *Biochem Soc Trans* 35(Pt 6):1479-84.

Braat H, Stokkers P, Hommes T, Cohn D, Vogels E, Pronk I, Spek A, van Kampen A, van Deventer S, Peppelenbosch M, and Hommes D (2005). Consequence of functional Nod2 and Tlr4 mutations on gene transcription in Crohn's disease patients. *J Mol Med* 83(8):601-9.

Braegger CP, Nicholls S, Murch SH, Stephens S, and MacDonald TT (1992). Tumour necrosis factor alpha in stool as a marker of intestinal inflammation. *Lancet* 339(8785):89-91.

Brand S, Staudinger T, Schnitzler F, Pfennig S, Hofbauer K, Dambacher J, Seiderer J, Tillack C, Konrad A, Crispin A, Goke B, Lohse P, and Ochsenkuhn T (2005). 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* 11(7):645-52.

Brant SR, Fu Y, Fields CT, Baltazar R, Ravenhill G, Pickles MR, Rohal PM, Mann J, Kirschner BS, Jabs EW, Bayless TM, Hanauer SB, and Cho JH (1998). American families with Crohn's disease have strong evidence for linkage to chromosome 16 but not chromosome 12. *Gastroenterology* 115(5):1056-61.

Brant SR, Panhuysen CI, Nicolae D, Reddy DM, Bonen DK, Karaliukas R, Zhang L, Swanson E, Datta LW, Moran T, Ravenhill G, Duerr RH, Achkar JP, Karban AS, and Cho JH (2003a). MDR1 Ala893 polymorphism is associated with inflammatory bowel disease. *Am J Hum Genet* 73(6):1282-92.

Brant SR, Picco MF, Achkar JP, Bayless TM, Kane SV, Brzezinski A, Nouvet FJ, Bonen D, Karban A, Dassopoulos T, Karaliukas R, Beaty TH, Hanauer SB, Duerr RH, and Cho JH (2003b). Defining complex contributions of NOD2/CARD15 gene mutations, age at onset, and tobacco use on Crohn's disease phenotypes. *Inflamm Bowel Dis* 9(5):281-9.

Bream JH, Carrington M, O'Toole S, Dean M, Gerrard B, Shin HD, Kosack D, Modi W, Young HA, and Smith MW (2000). Polymorphisms of the human IFNG gene noncoding regions. *Immunogenetics* 51(1):50-8.

Breese EJ, Michie CA, Nicholls SW, Murch SH, Williams CB, Domizio P, Walker-Smith JA, and MacDonald TT (1994). Tumor necrosis factor alpha-producing cells in the intestinal mucosa of children with inflammatory bowel disease. *Gastroenterology* 106(6):1455-66.

Browning BL, Huebner C, Petermann I, Demmers P, McCulloch A, Gearry RB, Barclay ML, Shelling AN, and Ferguson LR (2007). Association of DLG5 variants with inflammatory bowel disease in the New Zealand Caucasian population and meta-analysis of the DLG5 R30Q variant. *Inflamm Bowel Dis* 13(9):1069-76.

Browning BL, Annese V, Barclay ML, Bingham SA, Brand S, Buning 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, Muller-Myhsok B, Newman WG, Nimmo ER, Noble CL, Palmieri O, Parkes M, Petermann I, Rutgeerts P, Satsangi J, Shelling AN, Siminovitch KA, Torok HP, Tremelling M, Vermeire S, Valvano MR, and Witt H

(2008). Gender-stratified analysis of DLG5 R30Q in 4707 patients with Crohn disease and 4973 controls from 12 Caucasian cohorts. *J Med Genet* 45(1):36-42.

Buning C, Geerdts L, Fiedler T, Gentz E, Pitre G, Reuter W, Luck W, Buhner S, Molnar T, Nagy F, Lonovics J, Dignass A, Landt O, Nickel R, Genschel J, Lochs H, Schmidt HH, and Witt H (2006). DLG5 variants in inflammatory bowel disease. *Am J Gastroenterol* 101(4):786-92.

Buning C, Schmidt HH, Molnar T, De Jong DJ, Fiedler T, Buhner S, Sturm A, Baumgart DC, Nagy F, Lonovics J, Drenth JP, Landt O, Nickel R, Buttner J, Lochs H, and Witt H (2007). Heterozygosity for IL23R p.Arg381Gln confers a protective effect not only against Crohn's disease but also ulcerative colitis. *Aliment Pharmacol Ther* 26(7):1025-33.

Cao Z, Xiong J, Takeuchi M, Kurama T, and Goeddel DV (1996). TRAF6 is a signal transducer for interleukin-1. *Nature* 383(6599):443-6.

Cario E, and Podolsky DK (2000). Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. *Infect Immun* 68(12):7010-7.

Carter MJ, Lobo AJ, and Travis SP (2004). Guidelines for the management of inflammatory bowel disease in adults. *Gut* 53 Suppl 5:V1-16.

Cavanaugh J (2001). International collaboration provides convincing linkage replication in complex disease through analysis of a large pooled data set: Crohn disease and chromosome 16. *Am J Hum Genet* 68(5):1165-71.

Cavanaugh JA, Callen DF, Wilson SR, Stanford PM, Sraml ME, Gorska M, Crawford J, Whitmore SA, Shlegel C, Foote S, Kohonen-Corish M, and Pavli P (1998). Analysis of Australian Crohn's disease pedigrees refines the localization for susceptibility to inflammatory bowel disease on chromosome 16. *Ann Hum Genet* 62(Pt 4):291-8.

Chamaillard M, Philpott D, Girardin SE, Zouali H, Lesage S, Chareyre F, Bui TH, Giovannini M, Zaehringer U, Penard-Lacronique V, Sansonetti PJ, Hugot JP, and Thomas G (2003). Gene-environment interaction modulated by allelic heterogeneity in inflammatory diseases. *Proc Natl Acad Sci U S A* 100(6):3455-60.

Chen G, and Goeddel DV (2002). TNF-R1 signaling: a beautiful pathway. *Science* 296(5573):1634-5.

Chillon M, Casals T, Mercier B, Bassas L, Lissens W, Silber S, Romey MC, Ruiz-Romero J, Verlingue C, Claustres M, and et al. (1995). Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. *N Engl J Med* 332(22):1475-80.

Cho JH, Nicolae DL, Gold LH, Fields CT, LaBuda MC, Rohal PM, Pickles MR, Qin L, Fu Y, Mann JS, Kirschner BS, Jabs EW, Weber J, Hanauer SB, Bayless TM, and Brant SR (1998). Identification of novel susceptibility loci for inflammatory bowel disease on chromosomes 1p, 3q, and 4q: evidence for epistasis between 1p and IBD1. *Proc Natl Acad Sci U S A* 95(13):7502-7.

Cho JH, and Abraham C (2007). Inflammatory Bowel Disease Genetics: Nod2. *Annual Review of Medicine* 58(1):401-416.

Cho JH, and Weaver CT (2007). The genetics of inflammatory bowel disease. *Gastroenterology* 133(4):1327-39.

Chu CS, Trapnell BC, Murtagh JJ, Jr., Moss J, Dalemans W, Jallat S, Mercenier A, Pavirani A, Lecocq JP, Cutting GR, and et al. (1991). Variable deletion of exon 9 coding sequences in cystic fibrosis transmembrane conductance regulator gene mRNA transcripts in normal bronchial epithelium. *Embo J* 10(6):1355-63.

Chu CS, Trapnell BC, Curristin S, Cutting GR, and Crystal RG (1993). Genetic basis of variable exon 9 skipping in cystic fibrosis transmembrane conductance regulator mRNA. *Nat Genet* 3(2):151-6.

Cima RR, and Pemberton JH (2005). Medical and surgical management of chronic ulcerative colitis. *Arch Surg* 140(3):300-10.

Consortium MHCS (1999). Complete sequence and gene map of a human major histocompatibility complex. *Nature* 401(6756):921-923.

Consortium TWTCC (2007). Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 447(7145):661-78.

Coppin HL, Carmichael P, Lombardi G, L'Faqihi FE, Salter R, Parham P, Lechler RI, and de Preval C (1993). Position 71 in the alpha helix of the DR beta domain is predicted to influence peptide binding and plays a central role in allorecognition. *Eur J Immunol* 23(2):343-9.

Corrao G, Tragnone A, Caprilli R, Trallori G, Papi C, Andreoli A, Di Paolo M, Riegler G, Rigo GP, Ferrau O, Mansi C, Ingrosso M, and Valpiani D (1998). Risk of inflammatory bowel disease attributable to smoking, oral contraception and breastfeeding in Italy: a nationwide case-control study. Cooperative Investigators of the Italian Group for the Study of the Colon and the Rectum (GISC). *Int J Epidemiol* 27(3):397-404.

Coto-Segura P, Mallo-Garcia S, Costa-Romero M, Arostegui JI, Yague J, Ramos-Polo E, and Santos-Juanes J (2007). A sporadic case of early-onset sarcoidosis resembling Blau syndrome due to the recurrent R334W missense mutation on the NOD2 gene. *Br J Dermatol* 157(6):1257-9.

Creagh EM, and O'Neill LA (2006). TLRs, NLRs and RLRs: a trinity of pathogen sensors that co-operate in innate immunity. *Trends Immunol* 27(8):352-7.

Croucher PJ, Mascheretti S, Foelsch UR, Hampe J, and Schreiber S (2003a). Lack of association between the C3435T MDR1 gene polymorphism and inflammatory bowel disease in two independent Northern European populations. *Gastroenterology* 125(6):1919-20; author reply 1920-1.

Croucher PJ, Mascheretti S, Hampe J, Huse K, Frenzel H, Stoll M, Lu T, Nikolaus S, Yang SK, Krawczak M, Kim WH, and Schreiber S (2003b). Haplotype structure and association to Crohn's disease of CARD15 mutations in two ethnically divergent populations. *Eur J Hum Genet* 11(1):6-16.

Cucchiara S, Latiano A, Palmieri O, Canani RB, D'Inca R, Guariso G, Vieni G, De Venuto D, Riegler G, De'Angelis GL, Guagnozzi D, Bascietto C, Miele E, Valvano MR, Bossa F, and Annese V (2007a). Polymorphisms of tumor necrosis factor-alpha but not MDR1 influence response to medical therapy in pediatric-onset inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 44(2):171-9.

Cucchiara S, Latiano A, Palmieri O, Staiano AM, D'Inca R, Guariso G, Vieni G, Rutigliano V, Borrelli O, Valvano MR, and Annese V (2007b). Role of CARD15, DLG5 and OCTN genes polymorphisms in children with inflammatory bowel diseases. *World J Gastroenterol* 13(8):1221-9.

Cukovic-Cavka S, Vermeire S, Hrstic I, Claessens G, Kolacek S, Jakic-Razumovic J, Krznaric Z, Grubelic K, Radic D, Misak Z, Jadresin O, Rutgeerts P, and Vucelic B (2006). NOD2/CARD15 mutations in Croatian patients with Crohn's disease: prevalence and genotype-phenotype relationship. *Eur J Gastroenterol Hepatol* 18(8):895-9.

Cummings JR, Ahmad T, Geremia A, Beckly J, Cooney R, Hancock L, Pathan S, Guo C, Cardon LR, and Jewell DP (2007a). Contribution of the novel inflammatory bowel disease gene IL23R to disease susceptibility and phenotype. *Inflamm Bowel Dis* 13(9):1063-8.

Cummings JR, Cooney R, Pathan S, Anderson CA, Barrett JC, Beckly J, Geremia A, Hancock L, Guo C, Ahmad T, Cardon LR, and Jewell DP (2007b). Confirmation of the role of ATG16L1 as a Crohn's disease susceptibility gene. *Inflamm Bowel Dis* 13(8):941-6.

Curran ME, Lau KF, Hampe J, Schreiber S, Bridger S, Macpherson AJ, Cardon LR, Sakul H, Harris TJ, Stokkers P, Van Deventer SJ, Mirza M, Raedler A, Kruis W, Meckler U, Theuer D, Herrmann T, Gionchetti P, Lee J, Mathew C, and Lennard-Jones J (1998). Genetic analysis of inflammatory bowel disease in a large European cohort supports linkage to chromosomes 12 and 16. *Gastroenterology* 115(5):1066-71.

Cuthbert AP, Fisher SA, Mirza MM, King K, Hampe J, Roucher PJP, Mascheretti S, Sanderson J, Forbes A, Mansfield J, Schreiber S, Lewis CM, and Mathew CG (2002). The contribution of NOD2 gene mutations to the risk and site of disease in inflammatory bowel disease. *Gastroenterology* 122(4):867-874.

Daly MJ, Pearce AV, Farwell L, Fisher SA, Latiano A, Prescott NJ, Forbes A, Mansfield J, Sanderson J, Langelier D, Cohen A, Bitton A, Wild G, Lewis CM, Annese V, Mathew CG, and Rioux JD (2005). Association of DLG5 R30Q variant with inflammatory bowel disease. *Eur J Hum Genet* 13(7):835-9.

Davey MP, Martin TM, Planck SR, Lee J, Zamora D, and Rosenbaum JT (2006). Human endothelial cells express NOD2/CARD15 and increase IL-6 secretion in response to muramyl dipeptide. *Microvasc Res* 71(2):103-7.

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, and Rioux JD (2007). The role of the Toll receptor pathway in susceptibility to inflammatory bowel diseases. *Genes Immun* 8(5):387-97.

de la Concha EG, Fernandez-Arquero M, Martinez A, Vigil P, Vidal F, Lopez-Nava G, Diaz-Rubio M, and Garcia-Paredes J (1999). Amino acid polymorphism at residue 71 in HLA-DR beta chain plays a critical role in susceptibility to ulcerative colitis. *Dig Dis Sci* 44(11):2324-9.

de Saussure P, Clerson P, Prost PL, Truong Tan N, Bouhnik Y, and Gil R (2007). Appendectomy, smoking habits and the risk of developing ulcerative colitis: a case control study in private practice setting. *Gastroenterol Clin Biol* 31(5):493-7.

Dechairo B, Dimon C, van Heel D, Mackay I, Edwards M, Scambler P, Jewell D, Cardon L, Lench N, and Carey A (2001). Replication and extension studies of inflammatory bowel disease susceptibility regions confirm linkage to chromosome 6p (IBD3). *Eur J Hum Genet* 9(8):627-33.

Deng L, Wang C, Spencer E, Yang L, Braun A, You J, Slaughter C, Pickart C, and Chen ZJ (2000). Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell* 103(2):351-61.

Doyle SL, and O'Neill LA (2006). Toll-like receptors: from the discovery of NFkappaB to new insights into transcriptional regulations in innate immunity. *Biochem Pharmacol* 72(9):1102-13.

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, and Rotter JI (2007). IL-23 receptor (IL-23R) gene protects against pediatric Crohn's disease. *Inflamm Bowel Dis* 13(5):511-5.

Duerr RH, Barmada MM, Zhang L, Davis S, Preston RA, Chensny LJ, Brown JL, Ehrlich GD, Weeks DE, and Aston CE (1998). Linkage and association between inflammatory bowel disease and a locus on chromosome 12. *Am J Hum Genet* 63(1):95-100.

Duerr RH, Barmada MM, Zhang L, Pfutzer R, and Weeks DE (2000). High-density genome scan in Crohn disease shows confirmed linkage to chromosome 14q11-12. *Am J Hum Genet* 66(6):1857-62.

Duerr RH (2003). Update on the genetics of inflammatory bowel disease. *J Clin Gastroenterol* 37(5):358-67.

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, and Cho JH (2006). A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 314(5804):1461-3.

Economou M, Trikalinos TA, Loizou KT, Tsianos EV, and Ioannidis JP (2004). Differential effects of NOD2 variants on Crohn's disease risk and phenotype in diverse populations: a metaanalysis. *Am J Gastroenterol* 99(12):2393-404.

Ernst A, Jacobsen B, Ostergaard M, Okkels H, Andersen V, Dagiliene E, Pedersen IS, Thorsgaard N, Drewes AM, and Krarup HB (2007). Mutations in CARD15 and smoking confer susceptibility to Crohn's disease in the Danish population. *Scand J Gastroenterol*:1-7.

Erridge C, Stewart J, and Poxton IR (2003). Monocytes heterozygous for the Asp299Gly and Thr399Ile mutations in the Toll-like receptor 4 gene show no deficit in lipopolysaccharide signalling. *J Exp Med* 197(12):1787-91.

Farmer RG, Hawk WA, and Turnbull RB, Jr. (1975). Clinical patterns in Crohn's disease: a statistical study of 615 cases. *Gastroenterology* 68(4 Pt 1):627-35.

Farmer RG, Hawk WA, and Turnbull RB, Jr. (1976). Indications for surgery in Crohn's disease: analysis of 500 cases. *Gastroenterology* 71(2):245-50.

Farmer RG, Michener WM, and Mortimer EA (1980). Studies of family history among patients with inflammatory bowel disease. *Clin Gastroenterol* 9(2):271-7.

Farmer RG, Whelan G, and Fazio VW (1985). Long-term follow-up of patients with Crohn's disease. Relationship between the clinical pattern and prognosis. *Gastroenterology* 88(6):1818-25.

Farnood A, Naderi N, Moghaddam SJ, Noorinayer B, Firouzi F, Aghazadeh R, daryani NE, and Zali MR (2007). The frequency of C3435T MDR1 gene polymorphism in Iranian patients with ulcerative colitis. *Int J Colorectal Dis* 22(9):999-1003.

Farrokhyar F, Swarbrick ET, and Irvine EJ (2001). A critical review of epidemiological studies in inflammatory bowel disease. *Scand J Gastroenterol* 36(1):2-15.

Ferguson LR, Shelling AN, Browning BL, Huebner C, and Petermann I (2007). Genes, diet and inflammatory bowel disease. *Mutation Research* 622(1-2):70-83.

Fernandez L, Mendoza JL, Martinez A, Urcelay E, Fernandez-Arquero M, Garcia-Paredes J, Pena AS, Diaz-Rubio M, and de la Concha EG (2004). IBD1 and IBD3 determine location of Crohn's disease in the Spanish population. *Inflamm Bowel Dis* 10(6):715-22.

Ferraris A, Torres B, Knafelz D, Barabino A, Lionetti P, de Angelis GL, Iacono G, Papadatou B, D'Amato G, Di Ciommo V, Dallapiccola B, and Castro M (2006). Relationship between CARD15, SLC22A4/5, and DLG5 polymorphisms and early-onset inflammatory bowel diseases: an Italian multicentric study. *Inflamm Bowel Dis* 12(5):355-61.

Ferreira AC, Almeida S, Tavares M, Canedo P, Pereira F, Regalo G, Figueiredo C, Trindade E, Seruca R, Carneiro F, Amil J, Machado JC, and Tavarela-Veloso F (2005). NOD2/CARD15 and TNFA, but not IL1B and IL1RN, are associated with Crohn's disease. *Inflamm Bowel Dis* 11(4):331-9.

Firouzi F, Bahari A, Aghazadeh R, and Zali MR (2006). Appendectomy, tonsillectomy, and risk of inflammatory bowel disease: a case control study in Iran. *Int J Colorectal Dis* 21(2):155-9.

Fisher SA, Hampe J, Onnie CM, Daly MJ, Curley C, Purcell S, Sanderson J, Mansfield J, Annese V, Forbes A, Lewis CM, Schreiber S, Rioux JD, and Mathew CG (2006). Direct or indirect association in a

complex disease: the role of SLC22A4 and SLC22A5 functional variants in Crohn disease. *Hum Mutat* 27(8):778-85.

Franchi L, Park JH, Shaw MH, Marina-Garcia N, Chen G, Kim YG, and Nunez G (2008). Intracellular NOD-like receptors in innate immunity, infection and disease. *Cell Microbiol* 10(1):1-8.

Franchimont D, Vermeire S, El Housni H, Pierik M, Van Steen K, Gustot T, Quertinmont E, Abramowicz M, Van Gossum A, Deviere J, and Rutgeerts P (2004). Deficient host-bacteria interactions in inflammatory bowel disease? The toll-like receptor (TLR)-4 Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis. *Gut* 53(7):987-92.

Friedrichs F, Brescianini S, Annese V, Latiano A, Berger K, Kugathasan S, Broeckel U, Nikolaus S, Daly MJ, Schreiber S, Rioux JD, and Stoll M (2006). Evidence of transmission ratio distortion of DLG5 R30Q variant in general and implication of an association with Crohn disease in men. *Hum Genet* 119(3):305-11.

Gao M, Cao Q, Luo LH, Wu ML, Hu WL, and Si JM (2005). [NOD2/CARD15 gene polymorphisms and susceptibility to Crohn's disease in Chinese Han population]. *Zhonghua Nei Ke Za Zhi* 44(3):210-2.

Garcia Rodriguez LA, Gonzalez-Perez A, Johansson S, and Wallander MA (2005). Risk factors for inflammatory bowel disease in the general population. *Aliment Pharmacol Ther* 22(4):309-15.

Garland CF, Lilienfeld AM, Mendeloff AI, Markowitz JA, Terrell KB, and Garland FC (1981). Incidence rates of ulcerative colitis and Crohn's disease in fifteen areas of the United States. *Gastroenterology* 81(6):1115-24.

Gasche C, Scholmerich J, Brynskov J, D'Haens G, Hanauer SB, Irvine EJ, Jewell DP, Rachmilewitz D, Sachar DB, Sandborn WJ, and Sutherland LR (2000). A simple classification of Crohn's disease: report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998. *Inflamm Bowel Dis* 6(1):8-15.

Gazouli M, Zacharatos P, Gorgoulis V, Mantzaris G, Papalambros E, and Ikonomopoulos J (2004). The C3435T MDR1 gene polymorphism is not associated with susceptibility for ulcerative colitis in Greek population. *Gastroenterology* 126(1):367-9.

Gazouli M, Mantzaris G, Archimandritis AJ, Nasioulas G, and Anagnou NP (2005a). Single nucleotide polymorphisms of OCTN1, OCTN2, and DLG5 genes in Greek patients with Crohn's disease. *World J Gastroenterol* 11(47):7525-30.

Gazouli M, Mantzaris G, Kotsinas A, Zacharatos P, Papalambros E, Archimandritis A, Ikonomopoulos J, and Gorgoulis VG (2005b). Association between polymorphisms in the Toll-like receptor 4, CD14, and CARD15/NOD2 and inflammatory bowel disease in the Greek population. *World J Gastroenterol* 11(5):681-5.

Gearry RB, Lea RA, Roberts RL, Chambers GK, Barclay ML, and Kennedy MA (2006). CARD15 allele frequency differences in New Zealand Maori: ancestry specific susceptibility to Crohn's disease in New Zealand? *Gut* 55(4):580.

Giachino D, van Duist MM, Regazzoni S, Gregori D, Bardessono M, Salacone P, Scaglione N, Sostegni R, Sapone N, Bresso F, Sambataro A, Gaia E, Pera A, Astegiano M, and De Marchi M (2004). Analysis of the CARD15 variants R702W, G908R and L1007fs in Italian IBD patients. *Eur J Hum Genet* 12(3):206-12.

Giallourakis C, Stoll M, Miller K, Hampe J, Lander ES, Daly MJ, Schreiber S, and Rioux JD (2003). IBD5 is a general risk factor for inflammatory bowel disease: replication of association with Crohn disease and identification of a novel association with ulcerative colitis. *Am J Hum Genet* 73(1):205-11.

Giannini G, Ristori E, Cerignoli F, Rinaldi C, Zani M, Viel A, Ottini L, Crescenzi M, Martinotti S, Bignami M, Frati L, Screpanti I, and Gulino A (2002). Human MRE11 is inactivated in mismatch repair-deficient cancers. *EMBO Rep* 3(3):248-54.

Gilat T, Ribak J, Benaroya Y, Zemishlany Z, and Weissman I (1974). Ulcerative colitis in the Jewish population of Tel-Aviv Jafo. I. Epidemiology. *Gastroenterology* 66(3):335-42.

Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, Philpott DJ, and Sansonetti PJ (2003). Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J Biol Chem* 278(11):8869-72.

Glas J, Torok HP, Schiemann U, and Folwaczny C (2004). MDR1 gene polymorphism in ulcerative colitis. *Gastroenterology* 126(1):367.

Glas J, Seiderer J, Wetzke M, Konrad A, Torok 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, Goke B, Ochsenkuhn T, Muller-Myhsok B, Folwaczny M, Mussack T, and Brand S (2007). 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* 2(9):e819.

Glas J, Konrad A, Schmechel S, Dambacher J, Seiderer J, Schroff F, Wetzke M, Roeske D, Torok HP, Tonenchi L, Pfennig S, Haller D, Griga T, Klein W, Epplen JT, Folwaczny C, Lohse P, Goke B, Ochsenkuhn T, Mussack T, Folwaczny M, Muller-Myhsok B, and Brand S (2008). The ATG16L1 Gene Variants rs2241879 and rs2241880 (T300A) Are Strongly Associated With Susceptibility to Crohn's Disease in the German Population. *Am J Gastroenterol* 103(3):682-91.

Gleeson MH, Walker JS, Wentzel J, Chapman JA, and Harris R (1972). Human leucocyte antigens in Crohn's disease and ulcerative colitis. *Gut* 13(6):438-40.

Gonzalez-Mariscal L, Betanzos A, and Avila-Flores A (2000). MAGUK proteins: structure and role in the tight junction. *Semin Cell Dev Biol* 11(4):315-24.

Goyette P, Labbe C, Trinh TT, Xavier RJ, and Rioux JD (2007). Molecular pathogenesis of inflammatory bowel disease: genotypes, phenotypes and personalized medicine. *Ann Med* 39(3):177-99.

Gutierrez O, Pipaon C, Inohara N, Fontalba A, Ogura Y, Prosper F, Nunez G, and Fernandez-Luna JL (2002). Induction of Nod2 in myelomonocytic and intestinal epithelial cells via nuclear factor-kappa B activation. *J Biol Chem* 277(44):41701-5.

Halfvarson J, Bodin L, Tysk C, Lindberg E, and Jarnerot G (2003). Inflammatory bowel disease in a Swedish twin cohort: a long-term follow-up of concordance and clinical characteristics. *Gastroenterology* 124(7):1767-73.

Halfvarson J, Jess T, Magnuson A, Montgomery SM, Orholm M, Tysk C, Binder V, and Jarnerot G (2006). Environmental factors in inflammatory bowel disease: a co-twin control study of a Swedish-Danish twin population. *Inflamm Bowel Dis* 12(10):925-33.

Halme L, Turunen U, Helio T, Paavola P, Walle T, Miettinen A, Jarvinen H, Kontula K, and Farkkila M (2002). Familial and sporadic inflammatory bowel disease: comparison of clinical features and serological markers in a genetically homogeneous population. *Scand J Gastroenterol* 37(6):692-8.

Halme L, Paavola-Sakki P, Turunen U, Lappalainen M, Farkkila M, and Kontula K (2006). Family and twin studies in inflammatory bowel disease. *World J Gastroenterol* 12(23):3668-72.

Hampe J, Hermann B, Bridger S, MacPherson AJ, Mathew CG, and Schreiber S (1998). The interferon-gamma gene as a positional and functional candidate gene for inflammatory bowel disease. *Int J Colorectal Dis* 13(5-6):260-3.

Hampe J, Schreiber S, Shaw SH, Lau KF, Bridger S, Macpherson AJ, Cardon LR, Sakul H, Harris TJ, Buckler A, Hall J, Stokkers P, van Deventer SJ, Nurnberg P, Mirza MM, Lee JC, Lennard-Jones JE, Mathew CG, and Curran ME (1999a). A genomewide analysis provides evidence for novel linkages in inflammatory bowel disease in a large European cohort. *Am J Hum Genet* 64(3):808-16.

Hampe J, Shaw SH, Saiz R, Leysens N, Lantermann A, Mascheretti S, Lynch NJ, MacPherson AJ, Bridger S, van Deventer S, Stokkers P, Morin P, Mirza MM, Forbes A, Lennard-Jones JE, Mathew CG, Curran ME, and Schreiber S (1999b). Linkage of inflammatory bowel disease to human chromosome 6p. *Am J Hum Genet* 65(6):1647-55.

Hampe J, Cuthbert A, Croucher PJ, Mirza MM, Mascheretti S, Fisher S, Frenzel H, King K, Hasselmeyer A, MacPherson AJ, Bridger S, van Deventer S, Forbes A, Nikolaus S, Lennard-Jones JE, Foelsch UR, Krawczak M, Lewis C, Schreiber S, and Mathew CG (2001). Association between insertion mutation in NOD2 gene and Crohn's disease in German and British populations. *Lancet* 357(9272):1925-8.

Hampe J, Frenzel H, Mirza MM, Croucher PJ, Cuthbert A, Mascheretti S, Huse K, Platzer M, Bridger S, Meyer B, Nurnberg P, Stokkers P, Krawczak M, Mathew CG, Curran M, and Schreiber S (2002a). Evidence for a NOD2-independent susceptibility locus for inflammatory bowel disease on chromosome 16p. *Proc Natl Acad Sci U S A* 99(1):321-6.

Hampe J, Grebe J, Nikolaus S, Solberg C, Croucher PJ, Mascheretti S, Jahnsen J, Moum B, Klump B, Krawczak M, Mirza MM, Foelsch UR, Vatn M, and Schreiber S (2002b). Association of NOD2 (CARD 15) genotype with clinical course of Crohn's disease: a cohort study. *Lancet* 359(9318):1661-5.

Hampe J, Franke A, Rosenstiel P, Till A, Teuber M, Huse K, Albrecht M, Mayr G, De La Vega FM, Briggs J, Gunther S, Prescott NJ, Onnie CM, Hasler R, Sipos B, Folsch UR, Lengauer T, Platzer M, Mathew CG, Krawczak M, and Schreiber S (2007). A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nat Genet* 39(2):207-11.

Hanauer SB (1996). Inflammatory bowel disease. *N Engl J Med* 334(13):841-8.

Hanauer SB, and Sandborn W (2001). Management of Crohn's disease in adults. *Am J Gastroenterol* 96(3):635-43.

Hart AL, Al-Hassi HO, Rigby RJ, Bell SJ, Emmanuel AV, Knight SC, Kamm MA, and Stagg AJ (2005). Characteristics of Intestinal Dendritic Cells in Inflammatory Bowel Diseases. *Gastroenterology* 129(1):50-65.

Harvey RF, and Bradshaw JM (1980). A simple index of Crohn's-disease activity. *Lancet* 1(8167):514.

Hawkins CF (1990). Historical review. Inflammatory Bowel Diseases. R. N. Allan, M. R. B. Keighley, J. Alexander-Williams and C. F. Hawkins. Edinburgh, Churchill Livingstone**:** 3-9.

Heesen M, Bloemeke B, and Kunz D (2003). The cytokine synthesis by heterozygous carriers of the Toll-like receptor 4 Asp299Gly polymorphism does not differ from that of wild type homozygotes. *Eur Cytokine Netw* 14(4):234-7.

Henckaerts L, and Vermeire S (2007). NOD2/CARD15 disease associations other than Crohn's disease. *Inflamm Bowel Dis* 13(2):235-41.

Henriksen M, Jahnsen J, Lygren I, Sauar J, Kjellevold O, Schulz T, Vatn MH, and Moum B (2006). Ulcerative colitis and clinical course: results of a 5-year population-based follow-up study (the IBSEN study). *Inflamm Bowel Dis* 12(7):543-50.

Heresbach D, Gicquel-Douabin V, Birebent B, D'Halluin P N, Heresbach-Le Berre N, Dreano S, Siproudhis L, Dabadie A, Gosselin M, Mosser J, Semana G, Bretagne JF, and Yaouanq J (2004). NOD2/CARD15 gene polymorphisms in Crohn's disease: a genotype- phenotype analysis. *Eur J Gastroenterol Hepatol* 16(1):55-62.

Hirschhorn JN, and Daly MJ (2005). Genome-wide association studies for common diseases and complex traits. *Nat Rev Genet* 6(2):95-108.

Hisamatsu T, Suzuki M, Reinecker HC, Nadeau WJ, McCormick BA, and Podolsky DK (2003). CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. *Gastroenterology* 124(4):993-1000.

Ho GT, Nimmo ER, Tenesa A, Fennell J, Drummond H, Mowat C, Arnott ID, and Satsangi J (2005). Allelic variations of the multidrug resistance gene determine susceptibility and disease behavior in ulcerative colitis. *Gastroenterology* 128(2):288-96.

Ho GT, Soranzo N, Nimmo ER, Tenesa A, Goldstein DB, and Satsangi J (2006). ABCB1/MDR1 gene determines susceptibility and phenotype in ulcerative colitis: discrimination of critical variants using a gene-wide haplotype tagging approach. *Hum Mol Genet* 15(5):797-805.

Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A, Cascorbi I, Gerloff T, Roots I, Eichelbaum M, and Brinkmann U (2000). Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci U S A* 97(7):3473-8.

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, and Thomas G (1996). Mapping of a susceptibility locus for Crohn's disease on chromosome 16. *Nature* 379(6568):821-3.

Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, and Thomas G (2001). Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 411(6837):599-603.

Humbert P, Russell S, and Richardson H (2003). Dlg, Scribble and Lgl in cell polarity, cell proliferation and cancer. *Bioessays* 25(6):542-53.

Idestrom M, Rubio C, Granath F, Finkel Y, and Hugot JP (2005). CARD15 mutations are rare in Swedish pediatric Crohn disease. *J Pediatr Gastroenterol Nutr* 40(4):456-60.

Imahara SD, Jelacic S, Junker CE, and O'Keefe GE (2005). The TLR4 +896 polymorphism is not associated with lipopolysaccharide hypo-responsiveness in leukocytes. *Genes Immun* 6(1):37-43.

Inohara, Chamaillard, McDonald C, and Nunez G (2005). NOD-LRR proteins: role in host-microbial interactions and inflammatory disease. *Annu Rev Biochem* 74:355-83.

Inohara N, Koseki T, Lin J, del Peso L, Lucas PC, Chen FF, Ogura Y, and Nunez G (2000). An induced proximity model for NF-kappa B activation in the Nod1/RICK and RIP signaling pathways. *J Biol Chem* 275(36):27823-31.

Inohara N, Ogura Y, Fontalba A, Gutierrez O, Pons F, Crespo J, Fukase K, Inamura S, Kusumoto S, Hashimoto M, Foster SJ, Moran AP, Fernandez-Luna JL, and Nunez G (2003). Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. *J Biol Chem* 278(8):5509-12.

Inoue N, Tamura K, Kinouchi Y, Fukuda Y, Takahashi S, Ogura Y, Inohara N, Nunez G, Kishi Y, Koike Y, Shimosegawa T, Shimoyama T, and Hibi T (2002). Lack of common NOD2 variants in Japanese patients with Crohn's disease. *Gastroenterology* 123(1):86-91.

Irving PM, Shanahan F, and Rampton DS (2008). Drug Interactions in Inflammatory Bowel Disease. *The American Journal of Gastroenterology* 103(1):207-219.

Ismair MG, Vavricka SR, Kullak-Ublick GA, Fried M, Mengin-Lecreulx D, and Girardin SE (2006). hPepT1 selectively transports muramyl dipeptide but not Nod1-activating muramyl peptides. *Can J Physiol Pharmacol* 84(12):1313-9.

Jacobsen BA, Fallingborg J, Rasmussen HH, Nielsen KR, Drewes AM, Puho E, Nielsen GL, and Sorensen HT (2006). Increase in incidence and prevalence of inflammatory bowel disease in northern Denmark: a population-based study, 1978-2002. *Eur J Gastroenterol Hepatol* 18(6):601-6.

Jess T, Riis L, Jespersgaard C, Hougs L, Andersen PS, Orholm MK, Binder V, and Munkholm P (2005). Disease concordance, zygosity, and NOD2/CARD15 status: follow-up of a population-based cohort of Danish twins with inflammatory bowel disease. *Am J Gastroenterol* 100(11):2486-92.

Jess T, Riis L, Vind I, Winther KV, Borg S, Binder V, Langholz E, Thomsen OO, and Munkholm P (2007). Changes in clinical characteristics, course, and prognosis of inflammatory bowel disease during the last 5 decades: a population-based study from Copenhagen, Denmark. *Inflamm Bowel Dis* 13(4):481-9.

Jussila A, Tunturi-Hihnala H, and Färkkilä M (2004). Natural Course and Epidemiology of Crohn's disease in the Region of South Bothnia, Finland. *Gut* 53(Suppl VI):A140.

Kanazawa N, Matsushima S, Kambe N, Tachibana T, Nagai S, and Miyachi Y (2004). Presence of a Sporadic Case of Systemic Granulomatosis Syndrome with a CARD15 Mutation. 122(3):851-852.

Kanazawa N, Okafuji I, Kambe N, Nishikomori R, Nakata-Hizume M, Nagai S, Fuji A, Yuasa T, Manki A, Sakurai Y, Nakajima M, Kobayashi H, Fujiwara I, Tsutsumi H, Utani A, Nishigori C, Heike T, Nakahata T, and Miyachi Y (2005). Early-onset sarcoidosis and CARD15 mutations with constitutive nuclear factor-kappaB activation: common genetic etiology with Blau syndrome. *Blood* 105(3):1195-7.

Kanneganti TD, Lamkanfi M, and Nunez G (2007). Intracellular NOD-like receptors in host defense and disease. *Immunity* 27(4):549-59.

Karban A, Waterman M, Panhuysen CI, Pollak RD, Nesher S, Datta L, Weiss B, Suissa A, Shamir R, Brant SR, and Eliakim R (2004). NOD2/CARD15 genotype and phenotype differences between Ashkenazi and Sephardic Jews with Crohn's disease. *Am J Gastroenterol* 99(6):1134-40.

Karban A, Atia O, Leitersdorf E, Shahbari A, Sbeit W, Ackerman Z, Mualem R, Levine A, Nesher S, Safadi R, and Eliakim R (2005). The relation between NOD2/CARD15 mutations and the prevalence and phenotypic heterogeneity of Crohn's disease: lessons from the Israeli Arab Crohn's disease cohort. *Dig Dis Sci* 50(9):1692-7.

Karvonen A-L, Tunturi-Hihnala H, Krekelä I, Björknäs H, and Toivanen E (1993). Epidemiology of inflammatory bowel diseases in the region of Tampere University Hospital, Finland. *Scand J Gastroenterol* 28(Suppl 197):A77.

Kim RB, Leake BF, Choo EF, Dresser GK, Kubba SV, Schwarz UI, Taylor A, Xie HG, McKinsey J, Zhou S, Lan LB, Schuetz JD, Schuetz EG, and Wilkinson GR (2001). Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther* 70(2):189-99.

King K, Sheikh MF, Cuthbert AP, Fisher SA, Onnie CM, Mirza MM, Pattni RC, Sanderson J, Forbes A, Mansfield J, Lewis CM, Roberts RG, and Mathew CG (2006). Mutation, selection, and evolution of the Crohn disease susceptibility gene CARD15. *Hum Mutat* 27(1):44-54.

Kirsner JB, and Spencer JA (1963). Family Occurrences of Ulcerative Colitis, Regional Enteritis, and Ileocolitis. *Ann Intern Med* 59:133-44.

Klimecki WT, Futscher BW, Grogan TM, and Dalton WS (1994). P-glycoprotein expression and function in circulating blood cells from normal volunteers. *Blood* 83(9):2451-8.

Kobayashi KS, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nunez G, and Flavell RA (2005). Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science* 307(5710):731-4.

Komatsu M, Kobayashi D, Saito K, Furuya D, Yagihashi A, Araake H, Tsuji N, Sakamaki S, Niitsu Y, and Watanabe N (2001). Tumor necrosis factor-alpha in serum of patients with inflammatory bowel disease as measured by a highly sensitive immuno-PCR. *Clin Chem* 47(7):1297-301.

Kornbluth A, and Sachar DB (2004). Ulcerative colitis practice guidelines in adults (update): American College of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol* 99(7):1371-85.

Krawiec J, Odes HS, Lasry Y, Krugliak P, and Weitzman S (1984). Aspects of the epidemiology of Crohn's disease in the Jewish population in Beer Sheva, Israel. *Isr J Med Sci* 20(1):16-21.

Kuhbacher T, and Folsch UR (2007). Practical guidelines for the treatment of inflammatory bowel disease. *World J Gastroenterol* 13(8):1149-55.

Laharie D, Debeugny S, Peeters M, Van Gossum A, Gower-Rousseau C, Belaiche J, Fiasse R, Dupas JL, Lerebours E, Piotte S, Cortot A, Vermeire S, Grandbastien B, and Colombel JF (2001). Inflammatory bowel disease in spouses and their offspring. *Gastroenterology* 120(4):816-9.

Lakatos L, Lakatos PL, Willheim-Polli C, Reinisch W, Ferenci P, Tulassay Z, Molnar T, Kovacs A, Papp J, and Szalay F (2004). [NOD2/CARD15 mutations and genotype-phenotype correlations in patients with Crohn's disease. Hungarian multicenter study]. *Orv Hetil* 145(27):1403-11.

Lakatos PL, Lakatos L, Szalay F, Willheim-Polli C, Osterreicher C, Tulassay Z, Molnar T, Reinisch W, Papp J, Mozsik G, and Ferenci P (2005). Toll-like receptor 4 and NOD2/CARD15 mutations in Hungarian patients with Crohn's disease: phenotype-genotype correlations. *World J Gastroenterol* 11(10):1489-95.

Lakatos PL (2006). Recent trends in the epidemiology of inflammatory bowel diseases: up or down? *World J Gastroenterol* 12(38):6102-8.

Lakatos PL, Fischer S, Claes K, Kovacs A, Molnar T, Altorjay I, Demeter P, Tulassay Z, Palatka K, Papp M, Rutgeerts P, Szalay F, Papp J, Vermeire S, and Lakatos L (2006). DLG5 R30Q is not associated with IBD in Hungarian IBD patients but predicts clinical response to steroids in Crohn's disease. *Inflamm Bowel Dis* 12(5):362-8.

Lala S, Ogura Y, Osborne C, Hor SY, Bromfield A, Davies S, Ogunbiyi O, Nunez G, and Keshav S (2003). Crohn's disease and the NOD2 gene: a role for paneth cells. *Gastroenterology* 125(1):47-57.

Langholz E, Munkholm P, Davidsen M, and Binder V (1994). Course of ulcerative colitis: analysis of changes in disease activity over years. *Gastroenterology* 107(1):3-11.

Langmann T, Moehle C, Mauerer R, Scharl M, Liebisch G, Zahn A, Stremmel W, and Schmitz G (2004). Loss of detoxification in inflammatory bowel disease: dysregulation of pregnane X receptor target genes. *Gastroenterology* 127(1):26-40.

Lapidus A (2006). Crohn's disease in Stockholm County during 1990-2001: an epidemiological update. *World J Gastroenterol* 12(1):75-81.

Larson DW, and Pemberton JH (2004). Current concepts and controversies in surgery for IBD. *Gastroenterology* 126(6):1611-1619.

Latiano A, Palmieri O, Valvano RM, D'Inca R, Vecchi M, Ferraris A, Sturniolo GC, Spina L, Lombardi G, Dallapiccola B, Andriulli A, Devoto M, and Annese V (2006). Contribution of IBD5 locus to clinical features of IBD patients. *Am J Gastroenterol* 101(2):318-25.

Lecine P, Esmiol S, Metais J-Y, Nicoletti C, Nourry C, McDonald C, Nunez G, Hugot J-P, Borg J-P, and Ollendorff V (2007). The NOD2-RICK Complex Signals from the Plasma Membrane. *J. Biol. Chem.* 282(20):15197-15207.

Lee GH, Kim CG, Kim JS, Jung HC, and Song IS (2005). [Frequency analysis of NOD2 gene mutations in Korean patients with Crohn's disease]. *Korean J Gastroenterol* 45(3):162-8.

Lee JC, Lennard-Jones JE, and Cambridge G (1995). Antineutrophil antibodies in familial inflammatory bowel disease. *Gastroenterology* 108(2):428-33.

Lennard-Jones JE (1989). Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl* 170:2-6.

Leong RW, Armuzzi A, Ahmad T, Wong ML, Tse P, Jewell DP, and Sung JJ (2003). NOD2/CARD15 gene polymorphisms and Crohn's disease in the Chinese population. *Aliment Pharmacol Ther* 17(12):1465-70.

Lesage S, Zouali H, Cezard JP, Colombel JF, Belaiche J, Almer S, Tysk C, O'Morain C, Gassull M, Binder V, Finkel Y, Modigliani R, Gower-Rousseau C, Macry J, Merlin F, Chamaillard M, Jannot AS, Thomas G, and Hugot JP (2002). CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *Am J Hum Genet* 70(4):845-57.

Leung E, Hong J, Fraser AG, Merriman TR, Vishnu P, and Krissansen GW (2006). Polymorphisms in the organic cation transporter genes SLC22A4 and SLC22A5 and Crohn's disease in a New Zealand Caucasian cohort. *Immunol Cell Biol* 84(2):233-6.

Li J, Moran T, Swanson E, Julian C, Harris J, Bonen DK, Hedl M, Nicolae DL, Abraham C, and Cho JH (2004). Regulation of IL-8 and IL-1beta expression in Crohn's disease associated NOD2/CARD15 mutations. *Hum Mol Genet* 13(16):1715-25.

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, and Georges M (2007). Novel Crohn disease locus identified by genome-wide association maps to a gene desert on 5p13.1 and modulates expression of PTGER4. *PLoS Genet* 3(4):e58.

Lindberg E, Tysk C, Andersson K, and Jarnerot G (1988). Smoking and inflammatory bowel disease. A case control study. *Gut* 29(3):352-7.

Loftus CG, Loftus EV, Jr., Harmsen WS, Zinsmeister AR, Tremaine WJ, Melton LJ, 3rd, and Sandborn WJ (2007). Update on the incidence and prevalence of Crohn's disease and ulcerative colitis in Olmsted County, Minnesota, 1940-2000. *Inflamm Bowel Dis* 13(3):254-61.

Loftus EV, Jr., Silverstein MD, Sandborn WJ, Tremaine WJ, Harmsen WS, and Zinsmeister AR (1998). Crohn's disease in Olmsted County, Minnesota, 1940-1993: incidence, prevalence, and survival. *Gastroenterology* 114(6):1161-8.

Loftus EV, Jr., Silverstein MD, Sandborn WJ, Tremaine WJ, Harmsen WS, and Zinsmeister AR (2000). Ulcerative colitis in Olmsted County, Minnesota, 1940-1993: incidence, prevalence, and survival. *Gut* 46(3):336-43.

Loftus EV, Jr., Schoenfeld P, and Sandborn WJ (2002). The epidemiology and natural history of Crohn's disease in population-based patient cohorts from North America: a systematic review. *Aliment Pharmacol Ther* 16(1):51-60.

Loftus EV, Jr. (2004). Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology* 126(6):1504-17.

Logan RF, and Kay CR (1989). Oral contraception, smoking and inflammatory bowel disease--findings in the Royal College of General Practitioners Oral Contraception Study. *Int J Epidemiol* 18(1):105-7.

Ma Y, Ohmen JD, Li Z, Bentley LG, McElree C, Pressman S, Targan SR, Fischel-Ghodsian N, Rotter JI, and Yang H (1999). A genome-wide search identifies potential new susceptibility loci for Crohn's disease. *Inflamm Bowel Dis* 5(4):271-8.

Maeda S, Hsu LC, Liu H, Bankston LA, Iimura M, Kagnoff MF, Eckmann L, and Karin M (2005). Nod2 mutation in Crohn's disease potentiates NF-kappaB activity and IL-1beta processing. *Science* 307(5710):734-8.

Martin K, Radlmayr M, Borchers R, Heinzlmann M, and Folwaczny C (2002). Candidate genes colocalized to linkage regions in inflammatory bowel disease. *Digestion* 66(2):121-6.

Martinez A, Martin MC, Mendoza JL, Taxonera C, Diaz-Rubio M, de la Concha EG, and Urcelay E (2006). Association of the organic cation transporter OCTN genes with Crohn's disease in the Spanish population. *Eur J Hum Genet* 14(2):222-6.

Martinon F, and Tschopp J (2005). NLRs join TLRs as innate sensors of pathogens. *Trends Immunol* 26(8):447-54.

McGovern DP, Van Heel DA, Negoro K, Ahmad T, and Jewell DP (2003). Further evidence of IBD5/CARD15 (NOD2) epistasis in the susceptibility to ulcerative colitis. *Am J Hum Genet* 73(6):1465-6.

Medici V, Mascheretti S, Croucher PJ, Stoll M, Hampe J, Grebe J, Sturniolo GC, Solberg C, Jahnsen J, Moum B, Schreiber S, and Vatn MH (2006). Extreme heterogeneity in CARD15 and DLG5 Crohn disease-associated polymorphisms between German and Norwegian populations. *Eur J Hum Genet* 14(4):459-68.

Meinzer U, and Hugot JP (2005). Nod2 and Crohn's disease: many connected highways. *Lancet* 365(9473):1752-4.

Mendoza JL, Murillo LS, Fernandez L, Pena AS, Lana R, Urcelay E, Cruz-Santamaria DM, de la Concha EG, Diaz-Rubio M, and Garcia-Paredes J (2003). Prevalence of mutations of the NOD2/CARD15 gene and relation to phenotype in Spanish patients with Crohn disease. *Scand J Gastroenterol* 38(12):1235-40.

Miceli-Richard C, Lesage S, Rybojad M, Prieur AM, Manouvrier-Hanu S, Hafner R, Chamaillard M, Zouali H, Thomas G, and Hugot JP (2001). CARD15 mutations in Blau syndrome. *Nat Genet* 29(1):19-20.

Miceli-Richard C, Zouali H, Lesage S, Thomas G, Hugot JP, Said-Nahal R, and Breban M (2002). CARD15/NOD2 analyses in spondylarthropathy. *Arthritis Rheum* 46(5):1405-6.

Michel O, LeVan TD, Stern D, Dentener M, Thorn J, Gnat D, Beijer ML, Cochaux P, Holt PG, Martinez FD, and Rylander R (2003). Systemic responsiveness to lipopolysaccharide and polymorphisms in the toll-like receptor 4 gene in human beings. *J Allergy Clin Immunol* 112(5):923-9.

Mirza MM, Fisher SA, King K, Cuthbert AP, Hampe J, Sanderson J, Mansfield J, Donaldson P, Macpherson AJ, Forbes A, Schreiber S, Lewis CM, and Mathew CG (2003). Genetic evidence for interaction of the 5q31 cytokine locus and the CARD15 gene in Crohn disease. *Am J Hum Genet* 72(4):1018-22.

Mizushima N, Kuma A, Kobayashi Y, Yamamoto A, Matsubae M, Takao T, Natsume T, Ohsumi Y, and Yoshimori T (2003). Mouse Apg16L, a novel WD-repeat protein, targets to the autophagic isolation membrane with the Apg12-Apg5 conjugate. *J Cell Sci* 116(Pt 9):1679-88.

Molinie F, Gower-Rousseau C, Yzet T, Merle V, Grandbastien B, Marti R, Lerebours E, Dupas JL, Colombel JF, Salomez JL, and Cortot A (2004). Opposite evolution in incidence of Crohn's disease and ulcerative colitis in Northern France (1988-1999). *Gut* 53(6):843-8.

Monteleone G, Biancone L, Marasco R, Morrone G, Marasco O, Luzza F, and Pallone F (1997). Interleukin 12 is expressed and actively released by Crohn's disease intestinal lamina propria mononuclear cells. *Gastroenterology* 112(4):1169-78.

Munkholm P, Langholz E, Davidsen M, and Binder V (1995). Disease activity courses in a regional cohort of Crohn's disease patients. *Scand J Gastroenterol* 30(7):699-706.

Murch SH, Braegger CP, Walker-Smith JA, and MacDonald TT (1993). Location of tumour necrosis factor alpha by immunohistochemistry in chronic inflammatory bowel disease. *Gut* 34(12):1705-9.

Nakamura H, Sudo T, Tsuiki H, Miyake H, Morisaki T, Sasaki J, Masuko N, Kochi M, Ushio Y, and Saya H (1998). Identification of a novel human homolog of the Drosophila dlg, P-dlg, specifically expressed in the gland tissues and interacting with p55. *FEBS Lett* 433(1-2):63-7.

Negoro K, McGovern DP, Kinouchi Y, Takahashi S, Lench NJ, Shimosegawa T, Carey A, Cardon LR, Jewell DP, and van Heel DA (2003). Analysis of the IBD5 locus and potential gene-gene interactions in Crohn's disease. *Gut* 52(4):541-6.

Netea MG, Kullberg BJ, de Jong DJ, Franke B, Sprong T, Naber TH, Drenth JP, and Van der Meer JW (2004). NOD2 mediates anti-inflammatory signals induced by TLR2 ligands: implications for Crohn's disease. *Eur J Immunol* 34(7):2052-9.

Netea MG, Ferwerda G, de Jong DJ, Girardin SE, Kullberg BJ, and van der Meer JW (2005). NOD2 3020insC mutation and the pathogenesis of Crohn's disease: impaired IL-1beta production points to a loss-of-function phenotype. *Neth J Med* 63(8):305-8.

Neurath MF (2007). IL-23: a master regulator in Crohn disease. *Nat Med* 13(1):26-8.

Newman B, Silverberg MS, Gu X, Zhang Q, Lazaro A, Steinhart AH, Greenberg GR, Griffiths AM, McLeod RS, Cohen Z, Fernandez-Vina M, Amos CI, and Siminovitch K (2004). CARD15 and HLA DRB1 alleles influence susceptibility and disease localization in Crohn's disease. *Am J Gastroenterol* 99(2):306-15.

Newman WG, Gu X, Wintle RF, Liu X, van Oene M, Amos CI, and Siminovitch KA (2006). DLG5 variants contribute to Crohn disease risk in a Canadian population. *Hum Mutat* 27(4):353-8.

Nicholls S, Stephens S, Braegger CP, Walker-Smith JA, and MacDonald TT (1993). Cytokines in stools of children with inflammatory bowel disease or infective diarrhoea. *J Clin Pathol* 46(8):757-60.

Noble CL, Nimmo ER, Drummond H, Ho GT, Tenesa A, Smith L, Anderson N, Arnott ID, and Satsangi J (2005a). The contribution of OCTN1/2 variants within the IBD5 locus to disease susceptibility and severity in Crohn's disease. *Gastroenterology* 129(6):1854-64.

Noble CL, Nimmo ER, Drummond H, Smith L, Arnott ID, and Satsangi J (2005b). DLG5 variants do not influence susceptibility to inflammatory bowel disease in the Scottish population. *Gut* 54(10):1416-20.

Norio R (2003). Finnish Disease Heritage II: population prehistory and genetic roots of Finns. *Hum Genet* 112(5-6):457-69.

Nunez C, Barreiro M, Dominguez-Munoz JE, Lorenzo A, Zapata C, and Pena AS (2004). CARD15 mutations in patients with Crohn's disease in a homogeneous Spanish population. *Am J Gastroenterol* 99(3):450-6.

Odes HS, Fraser D, and Krawiec J (1987a). Incidence of idiopathic ulcerative colitis in Jewish population subgroups in the Beer Sheva region of Israel. *Am J Gastroenterol* 82(9):854-8.

Odes HS, Fraser D, and Krawiec J (1987b). Ulcerative colitis in the Jewish population of southern Israel 1961-1985: epidemiological and clinical study. *Gut* 28(12):1630-6.

Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nunez G, and Cho JH (2001a). A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 411(6837):603-6.

Ogura Y, Inohara N, Benito A, Chen FF, Yamaoka S, and Nunez G (2001b). Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB. *J Biol Chem* 276(7):4812-8.

Ogura Y, Lala S, Xin W, Smith E, Dowds TA, Chen FF, Zimmermann E, Tretiakova M, Cho JH, Hart J, Greenson JK, Keshav S, and Nunez G (2003). Expression of NOD2 in Paneth cells: a possible link to Crohn's ileitis. *Gut* 52(11):1591-7.

Ohmen JD, Yang HY, Yamamoto KK, Zhao HY, Ma Y, Bentley LG, Huang Z, Gerwehr S, Pressman S, McElree C, Targan S, Rotter JI, and Fischel-Ghodsian N (1996). Susceptibility locus for inflammatory bowel disease on chromosome 16 has a role in Crohn's disease, but not in ulcerative colitis. *Hum Mol Genet* 5(10):1679-83.

Oliver J, Rueda B, Lopez-Nevot MA, Gomez-Garcia M, and Martin J (2007). Replication of an Association Between IL23R Gene Polymorphism With Inflammatory Bowel Disease. *Clin Gastroenterol Hepatol* 5(8):977-981 e2.

Onnie C, Fisher SA, King K, Mirza M, Roberts R, Forbes A, Sanderson J, Lewis CM, and Mathew CG (2006a). Sequence variation, linkage disequilibrium and association with Crohn's disease on chromosome 5q31. *Genes Immun* 7(5):359-65.

Onnie CM, Fisher SA, Pattni R, Sanderson J, Forbes A, Lewis CM, and Mathew CG (2006b). Associations of allelic variants of the multidrug resistance gene (ABCB1 or MDR1) and inflammatory bowel disease and their effects on disease behavior: a case-control and meta-analysis study. *Inflamm Bowel Dis* 12(4):263-71.

Oostenbrug LE, Drenth JP, de Jong DJ, Nolte IM, Oosterom E, van Dullemen HM, van der Linde K, te Meerman GJ, van der Steege G, Kleibeuker JH, and Jansen PL (2005). Association between Toll-like receptor 4 and inflammatory bowel disease. *Inflamm Bowel Dis* 11(6):567-75.

Oostenbrug LE, Dijkstra G, Nolte IM, van Dullemen HM, Oosterom E, Faber KN, de Jong DJ, van der Linde K, te Meerman GJ, van der Steege G, Kleibeuker JH, and Jansen PL (2006a). Absence of association between the multidrug resistance (MDR1) gene and inflammatory bowel disease. *Scand J Gastroenterol* 41(10):1174-82.

Oostenbrug LE, Nolte IM, Oosterom E, van der Steege G, te Meerman GJ, van Dullemen HM, Drenth JP, de Jong DJ, van der Linde K, Jansen PL, and Kleibeuker JH (2006b). CARD15 in inflammatory bowel disease and Crohn's disease phenotypes: an association study and pooled analysis. *Dig Liver Dis* 38(11):834-45.

Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, Vega F, Yu N, Wang J, Singh K, Zonin F, Vaisberg E, Churakova T, Liu M, Gorman D, Wagner J, Zurawski S, Liu Y, Abrams JS, Moore KW, Rennick D, de Waal-Malefyt R, Hannum C, Bazan JF, and Kastelein RA (2000). Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* 13(5):715-25.

Orchard TR, Satsangi J, Van Heel D, and Jewell DP (2000). Genetics of inflammatory bowel disease: a reappraisal. *Scand J Immunol* 51(1):10-7.

Orholm M, Munkholm P, Langholz E, Nielsen OH, Sorensen TI, and Binder V (1991). Familial occurrence of inflammatory bowel disease. *N Engl J Med* 324(2):84-8.

Orholm M, Binder V, Sorensen TI, Rasmussen LP, and Kyvik KO (2000). Concordance of inflammatory bowel disease among Danish twins. Results of a nationwide study. *Scand J Gastroenterol* 35(10):1075-81.

Osuga T, Sakaeda T, Nakamura T, Yamada T, Koyama T, Tamura T, Aoyama N, Okamura N, Kasuga M, and Okumura K (2006). MDR1 C3435T polymorphism is predictive of later onset of ulcerative colitis in Japanese. *Biol Pharm Bull* 29(2):324-9.

Otte JM, Rosenberg IM, and Podolsky DK (2003). Intestinal myofibroblasts in innate immune responses of the intestine. *Gastroenterology* 124(7):1866-78.

Ouburg S, Mallant-Hent R, Crusius JB, van Bodegraven AA, Mulder CJ, Linskens R, Pena AS, and Morre SA (2005). 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* 54(3):439-40.

Ouyang Q, Tandon R, Goh KL, Ooi CJ, Ogata H, and Fiocchi C (2005). The emergence of inflammatory bowel disease in the Asian Pacific region. *Curr Opin Gastroenterol* 21(4):408-13.

Paavola-Sakki P, Ollikainen V, Helio T, Halme L, Turunen U, Lahermo P, Lappalainen M, Farkkila M, and Kontula K (2003). Genome-wide search in Finnish families with inflammatory bowel disease provides evidence for novel susceptibility loci. *Eur J Hum Genet* 11(2):112-20.

Palmieri O, Latiano A, Valvano R, D'Inca R, Vecchi M, Sturniolo GC, Saibeni S, Bossa F, Latiano T, Devoto M, Andriulli A, and Annese V (2005). Multidrug resistance 1 gene polymorphisms are not associated with inflammatory bowel disease and response to therapy in Italian patients. *Aliment Pharmacol Ther* 22(11-12):1129-38.

Panwala CM, Jones JC, and Viney JL (1998). A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, mdr1a, spontaneously develop colitis. *J Immunol* 161(10):5733-44.

Parham C, Chirica M, Timans J, Vaisberg E, Travis M, Cheung J, Pflanz S, Zhang R, Singh KP, Vega F, To W, Wagner J, O'Farrell A-M, McClanahan T, Zurawski S, Hannum C, Gorman D, Rennick DM, Kastelein RA, de Waal Malefyt R, and Moore KW (2002). A Receptor for the Heterodimeric Cytokine IL-23 Is Composed of IL-12R{beta}1 and a Novel Cytokine Receptor Subunit, IL-23R. *J Immunol* 168(11):5699-5708.

Parkes M, Barmada MM, Satsangi J, Weeks DE, Jewell DP, and Duerr RH (2000). The IBD2 locus shows linkage heterogeneity between ulcerative colitis and Crohn disease. *Am J Hum Genet* 67(6):1605-10.

Parrello T, Pavia M, Angelillo IF, Monteleone G, Riegler G, Papi G, D'Inca R, Annese V, Tonelli F, Caprilli R, and Pallone F (1997). Appendectomy is an independent protective factor for ulcerative colitis: results of a multicentre case control study. The Italian Group for the Study of the Colon and Rectum (GISC). *Ital J Gastroenterol Hepatol* 29(3):208-11.

Parronchi P, Romagnani P, Annunziato F, Sampognaro S, Becchio A, Giannarini L, Maggi E, Pupilli C, Tonelli F, and Romagnani S (1997). Type 1 T-helper cell predominance and interleukin-12 expression in the gut of patients with Crohn's disease. *Am J Pathol* 150(3):823-32.

Pauleau AL, and Murray PJ (2003). Role of nod2 in the response of macrophages to toll-like receptor agonists. *Mol Cell Biol* 23(21):7531-9.

Pearce AV, Fisher SA, Prescott NJ, Onnie CM, Pattni R, Green P, Forbes A, Mansfield J, Sanderson J, Schreiber S, Lewis CM, and Mathew CG (2007). Investigation of association of the DLG5 gene with phenotypes of inflammatory bowel disease in the British population. *Int J Colorectal Dis* 22(4):419-24.

Peeters M, Nevens H, Baert F, Hiele M, de Meyer AM, Vlietinck R, and Rutgeerts P (1996). Familial aggregation in Crohn's disease: increased age-adjusted risk and concordance in clinical characteristics. *Gastroenterology* 111(3):597-603.

Peltekova VD, Wintle RF, Rubin LA, Amos CI, Huang Q, Gu X, Newman B, Van Oene M, Cescon D, Greenberg G, Griffiths AM, St George-Hyslop PH, and Siminovitch KA (2004). Functional variants of OCTN cation transporter genes are associated with Crohn disease. *Nat Genet* 36(5):471-5.

Pena AS, and Wijmenga C (2001). Genetic factors underlying gluten-sensitive enteropathy. *Curr Allergy Asthma Rep* 1(6):526-33.

Perencevich M, and Burakoff R (2006). Use of antibiotics in the treatment of inflammatory bowel disease. *Inflamm Bowel Dis* 12(7):651-64.

Philpott DJ, and Girardin SE (2004). The role of Toll-like receptors and Nod proteins in bacterial infection. *Mol Immunol* 41(11):1099-108.

Philpott DJ, and Viala J (2004). Towards an understanding of the role of NOD2/CARD15 in the pathogenesis of Crohn's disease. *Best Pract Res Clin Gastroenterol* 18(3):555-68.

Pierik M, Vermeire S, Steen KV, Joossens S, Claessens G, Vlietinck R, and Rutgeerts P (2004). Tumour necrosis factor-alpha receptor 1 and 2 polymorphisms in inflammatory bowel disease and their association with response to infliximab. *Aliment Pharmacol Ther* 20(3):303-10.

Polito JM, 2nd, Childs B, Mellits ED, Tokayer AZ, Harris ML, and Bayless TM (1996a). Crohn's disease: influence of age at diagnosis on site and clinical type of disease. *Gastroenterology* 111(3):580-6.

Polito JM, 2nd, Rees RC, Childs B, Mendeloff AI, Harris ML, and Bayless TM (1996b). Preliminary evidence for genetic anticipation in Crohn's disease. *Lancet* 347(9004):798-800.

Potocnik U, Ferkolj I, Glavac D, and Dean M (2004). Polymorphisms in multidrug resistance 1 (MDR1) gene are associated with refractory Crohn disease and ulcerative colitis. *Genes Immun* 5(7):530-9.

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, and Mathew CG (2007). A nonsynonymous SNP in ATG16L1 predisposes to ileal Crohn's disease and is independent of CARD15 and IBD5. *Gastroenterology* 132(5):1665-71.

Quinton JF, Sendid B, Reumaux D, Duthilleul P, Cortot A, Grandbastien B, Charrier G, Targan SR, Colombel JF, and Poulain D (1998). Anti-Saccharomyces cerevisiae mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. *Gut* 42(6):788-91.

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, and Schreiber S (2007). Genome-wide association study for Crohn's disease in the Quebec Founder Population identifies multiple validated disease loci. *Proc Natl Acad Sci U S A* 104(37):14747-52.

Riis L, Vind I, Vermeire S, Wolters F, Katsanos K, Politi P, Freitas J, Mouzas IA, O'Morain C, Ruiz-Ochoa V, Odes S, Binder V, Munkholm P, Moum B, Stockbrugger R, and Langholz E (2007). The prevalence of genetic and serologic markers in an unselected European population-based cohort of IBD patients. *Inflamm Bowel Dis* 13(1):24-32.

Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, McLeod RS, Griffiths AM, Green T, Brettin TS, Stone V, Bull SB, Bitton A, Williams CN, Greenberg GR, Cohen Z, Lander ES, Hudson TJ, and Siminovitch KA (2000). Genomewide search in Canadian families with inflammatory bowel disease reveals two novel susceptibility loci. *Am J Hum Genet* 66(6):1863-70.

Rioux JD, Daly MJ, Silverberg MS, Lindblad K, Steinhart H, Cohen Z, Delmonte T, Kocher K, Miller K, Guschwan S, Kulbokas EJ, O'Leary S, Winchester E, Dewar K, Green T, Stone V, Chow C, Cohen A, Langelier D, Lapointe G, Gaudet D, Faith J, Branco N, Bull SB, McLeod RS, Griffiths AM, Bitton A, Greenberg GR, Lander ES, Siminovitch KA, and Hudson TJ (2001). Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. *Nat Genet* 29(2):223-8.

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, and Brant SR (2007). Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat Genet* 39(5):596-604.

Roberts RL, Gearry RB, Hollis-Moffatt JE, Miller AL, Reid J, Abkevich V, Timms KM, Gutin A, Lanchbury JS, Merriman TR, Barclay ML, and Kennedy MA (2007). IL23R R381Q and ATG16L1 T300A are strongly associated with Crohn's disease in a study of New Zealand Caucasians with inflammatory bowel disease. *Am J Gastroenterol* 102(12):2754-61.

Rogler G, Brand K, Vogl D, Page S, Hofmeister R, Andus T, Knuechel R, Baeuerle PA, Scholmerich J, and Gross V (1998). Nuclear factor kappaB is activated in macrophages and epithelial cells of inflamed intestinal mucosa. *Gastroenterology* 115(2):357-69.

Roth MP, Petersen GM, McElree C, Feldman E, and Rotter JI (1989). Geographic origins of Jewish patients with inflammatory bowel disease. *Gastroenterology* 97(4):900-4.

Rothfuss KS, Stange EF, and Herrlinger KR (2006). Extraintestinal manifestations and complications in inflammatory bowel diseases. *World J Gastroenterol* 12(30):4819-31.

Roussomoustakaki M, Satsangi J, Welsh K, Louis E, Fanning G, Targan S, Landers C, and Jewell DP (1997). Genetic markers may predict disease behavior in patients with ulcerative colitis. *Gastroenterology* 112(6):1845-1853.

Rozen P, Zonis J, Yekutiel P, and Gilat T (1979). Crohn's disease in the Jewish population of Tel-Aviv-Yafo. Epidemiologic and clinical aspects. *Gastroenterology* 76(1):25-30.

Russel MG, and Stockbrugger RW (1996). Epidemiology of inflammatory bowel disease: an update. *Scand J Gastroenterol* 31(5):417-27.

Russel MG (2000). Changes in the incidence of inflammatory bowel disease: what does it mean? *Eur J Intern Med* 11(4):191-196.

Russell RK, and Satsangi J (2004). IBD: a family affair. *Best Pract Res Clin Gastroenterol* 18(3):525-39.

Russell RK, Drummond HE, Nimmo ER, Anderson NH, Noble CL, Wilson DC, Gillett PM, McGrogan P, Hassan K, Weaver LT, Bisset WM, Mahdi G, and Satsangi J (2006). Analysis of the influence of OCTN1/2 variants within the IBD5 locus on disease susceptibility and growth indices in early onset inflammatory bowel disease. *Gut* 55(8):1114-23.

Russell RK, Drummond HE, Nimmo ER, Anderson N, Wilson DC, Gillett PM, McGrogan P, Hassan K, Weaver LT, Bisset WM, Mahdi G, and Satsangi J (2007). The contribution of the DLG5 113A variant in early-onset inflammatory bowel disease. *J Pediatr* 150(3):268-73.

Rutgeerts P, D'Haens G, Hiele M, Geboes K, and Vantrappen G (1994). Appendectomy protects against ulcerative colitis. *Gastroenterology* 106(5):1251-3.

Sands BE (2007). Inflammatory bowel disease: past, present, and future. *J Gastroenterol* 42(1):16-25.

Sashio H, Tamura K, Ito R, Yamamoto Y, Bamba H, Kosaka T, Fukui S, Sawada K, Fukuda Y, Satomi M, Shimoyama T, and Furuyama J (2002). Polymorphisms of the TNF gene and the TNF receptor superfamily member 1B gene are associated with susceptibility to ulcerative colitis and Crohn's disease, respectively. *Immunogenetics* 53(12):1020-7.

Satsangi J, Jewell DP, Rosenberg WM, and Bell JI (1994). Genetics of inflammatory bowel disease. *Gut* 35(5):696-700.

Satsangi J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell JI, and Jewell DP (1996a). Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. *Nat Genet* 14(2):199-202.

Satsangi J, Welsh KI, Bunce M, Julier C, Farrant JM, Bell JI, and Jewell DP (1996b). Contribution of genes of the major histocompatibility complex to susceptibility and disease phenotype in inflammatory bowel disease. *Lancet* 347(9010):1212-7.

Satsangi J, Jewell DP, and Bell JI (1997). The genetics of inflammatory bowel disease. *Gut* 40(5):572-4.

Schmid D, Dengjel J, Schoor O, Stevanovic S, and Munz C (2006). Autophagy in innate and adaptive immunity against intracellular pathogens. *J Mol Med* 84(3):194-202.

Schnitzler F, Brand S, Staudinger T, Pfennig S, Hofbauer K, Seiderer J, Tillack C, Goke B, Ochsenkuhn T, and Lohse P (2006). Eight novel CARD15 variants detected by DNA sequence analysis of the CARD15 gene in 111 patients with inflammatory bowel disease. *Immunogenetics* 58(2-3):99-106.

Schreiber S, Nikolaus S, and Hampe J (1998). Activation of nuclear factor kappa B inflammatory bowel disease. *Gut* 42(4):477-84.

Schwab M, Schaeffeler E, Marx C, Fromm MF, Kaskas B, Metzler J, Stange E, Herfarth H, Schoelmerich J, Gregor M, Walker S, Cascorbi I, Roots I, Brinkmann U, Zanger UM, and Eichelbaum M (2003). Association between the C3435T MDR1 gene polymorphism and susceptibility for ulcerative colitis. *Gastroenterology* 124(1):26-33.

Schwartz DA (2002). The genetics of innate immunity. *Chest* 121(3 Suppl):62S-68S.

Seibold F, Slametschka D, Gregor M, and Weber P (1994). Neutrophil autoantibodies: a genetic marker in primary sclerosing cholangitis and ulcerative colitis. *Gastroenterology* 107(2):532-6.

Seibold F, Stich O, Hufnagl R, Kamil S, and Scheurlen M (2001). Anti-Saccharomyces cerevisiae antibodies in inflammatory bowel disease: a family study. *Scand J Gastroenterol* 36(2):196-201.

Shanahan F, Duerr RH, Rotter JI, Yang H, Sutherland LR, McElree C, Landers CJ, and Targan SR (1992). Neutrophil autoantibodies in ulcerative colitis: familial aggregation and genetic heterogeneity. *Gastroenterology* 103(2):456-61.

Shaulian E, and Karin M (2001). AP-1 in cell proliferation and survival. *Oncogene* 20(19):2390-400.

Shealy DJ, and Visvanathan S (2008). Anti-TNF antibodies: lessons from the past, roadmap for the future. *Handb Exp Pharmacol*(181):101-29.

Shivananda S, Lennard-Jones J, Logan R, Fear N, Price A, Carpenter L, and van Blankenstein M (1996). Incidence of inflammatory bowel disease across Europe: is there a difference between north and south? Results of the European Collaborative Study on Inflammatory Bowel Disease (EC-IBD). *Gut* 39(5):690-7.

Silverberg MS, Mirea L, Bull SB, Murphy JE, Steinhart AH, Greenberg GR, McLeod RS, Cohen Z, Wade JA, and Siminovitch KA (2003). A population- and family-based study of Canadian families reveals association of HLA DRB1*0103 with colonic involvement in inflammatory bowel disease. *Inflamm Bowel Dis* 9(1):1-9.

Silverberg MS, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, Caprilli R, Colombel JF, Gasche C, Geboes K, Jewell DP, Karban A, Loftus Jr EV, Pena AS, Riddell RH, Sachar DB, Schreiber S, Steinhart AH, Targan SR, Vermeire S, and Warren BF (2005). Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 19 Suppl A:5-36.

Silverberg MS, Duerr RH, Brant SR, Bromfield G, Datta LW, Jani N, Kane SV, Rotter JI, Philip Schumm L, Hillary Steinhart A, Taylor KD, Yang H, Cho JH, Rioux JD, and Daly MJ (2007). Refined genomic localization and ethnic differences observed for the IBD5 association with Crohn's disease. *Eur J Hum Genet* 15(3):328-35.

Silverstein MD, Lashner BA, Hanauer SB, Evans AA, and Kirsner JB (1989). Cigarette smoking in Crohn's disease. *Am J Gastroenterol* 84(1):31-3.

Simmons JD, Mullighan C, Welsh KI, and Jewell DP (2000). Vitamin D receptor gene polymorphism: association with Crohn's disease susceptibility. *Gut* 47(2):211-4.

Sipponen T, Savilahti E, Kolho KL, Nuutinen H, Turunen U, and Farkkila M (2008). Crohn's disease activity assessed by fecal calprotectin and lactoferrin: correlation with Crohn's disease activity index and endoscopic findings. *Inflamm Bowel Dis* 14(1):40-6.

Sonnenberg A, McCarty DJ, and Jacobsen SJ (1991). Geographic variation of inflammatory bowel disease within the United States. *Gastroenterology* 100(1):143-9.

Sonnenberg A, and Wasserman IH (1991). Epidemiology of inflammatory bowel disease among U.S. military veterans. *Gastroenterology* 101(1):122-30.

Steinman L (2007). A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. *Nat Med* 13(2):139-45.

Stokkers PC, Reitsma PH, Tytgat GN, and van Deventer SJ (1999). HLA-DR and -DQ phenotypes in inflammatory bowel disease: a meta-analysis. *Gut* 45(3):395-401.

Stoll M, Corneliussen B, Costello CM, Waetzig GH, Mellgard B, Koch WA, Rosenstiel P, Albrecht M, Croucher PJ, Seegert D, Nikolaus S, Hampe J, Lengauer T, Pierrou S, Foelsch UR, Mathew CG, Lagerstrom-Fermer M, and Schreiber S (2004). Genetic variation in DLG5 is associated with inflammatory bowel disease. *Nat Genet* 36(5):476-80.

Strober W, Murray PJ, Kitani A, and Watanabe T (2006). Signalling pathways and molecular interactions of NOD1 and NOD2. *Nat Rev Immunol* 6(1):9-20.

Sugimura K, Taylor KD, Lin YC, Hang T, Wang D, Tang YM, Fischel-Ghodsian N, Targan SR, Rotter JI, and Yang H (2003a). A novel NOD2/CARD15 haplotype conferring risk for Crohn disease in Ashkenazi Jews. *Am J Hum Genet* 72(3):509-18.

Sugimura M, Kinouchi Y, Takahashi S, Aihara H, Takagi S, Negoro K, Obana N, Kojima Y, Matsumoto K, Kikuchi T, Hiroki M, Oomori S, and Shimosegawa T (2003b). CARD15/NOD2 mutational analysis in Japanese patients with Crohn's disease. *Clin Genet* 63(2):160-2.

Sun L, Roesler J, Rosen-Wolff A, Winkler U, Koch R, Thurigen A, and Henker J (2003). CARD15 genotype and phenotype analysis in 55 pediatric patients with Crohn disease from Saxony, Germany. *J Pediatr Gastroenterol Nutr* 37(4):492-7.

Sutherland L, Singleton J, Sessions J, Hanauer S, Krawitt E, Rankin G, Summers R, Mekhjian H, Greenberger N, Kelly M, and et al. (1991). Double blind, placebo controlled trial of metronidazole in Crohn's disease. *Gut* 32(9):1071-5.

Sutton CL, Yang H, Li Z, Rotter JI, Targan SR, and Braun J (2000). Familial expression of anti-Saccharomyces cerevisiae mannan antibodies in affected and unaffected relatives of patients with Crohn's disease. *Gut* 46(1):58-63.

Tabor HK, Risch NJ, and Myers RM (2002). Candidate-gene approaches for studying complex genetic traits: practical considerations. *Nat Rev Genet* 3(5):391-7.

Tanabe M, Ieiri I, Nagata N, Inoue K, Ito S, Kanamori Y, Takahashi M, Kurata Y, Kigawa J, Higuchi S, Terakawa N, and Otsubo K (2001). Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. *J Pharmacol Exp Ther* 297(3):1137-43.

Tanabe T, Chamaillard M, Ogura Y, Zhu L, Qiu S, Masumoto J, Ghosh P, Moran A, Predergast MM, Tromp G, Williams CJ, Inohara N, and Nunez G (2004). Regulatory regions and critical residues of NOD2 involved in muramyl dipeptide recognition. *EMBO J* 23(7):1587-97.

Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, and Willingham MC (1987). Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci U S A* 84(21):7735-8.

Thjodleifsson B, Sigthorsson G, Cariglia N, Reynisdottir I, Gudbjartsson DF, Kristjansson K, Meddings JB, Gudnason V, Wandall JH, Andersen LP, Sherwood R, Kjeld M, Oddsson E, Gudjonsson H, and Bjarnason I (2003). Subclinical intestinal inflammation: an inherited abnormality in Crohn's disease relatives? *Gastroenterology* 124(7):1728-1737.

Thompson NP, Driscoll R, Pounder RE, and Wakefield AJ (1996). Genetics versus environment in inflammatory bowel disease: results of a British twin study. *Bmj* 312(7023):95-6.

Tobin MV, Logan RF, Langman MJ, McConnell RB, and Gilmore IT (1987). Cigarette smoking and inflammatory bowel disease. *Gastroenterology* 93(2):316-21.

Todd JA, and Farrall M (1996). Panning for gold: genome-wide scanning for linkage in type 1 diabetes. *Hum Mol Genet* 5 Spec No:1443-8.

Torkvist L, Noble CL, Lordal M, Sjoqvist U, Lindforss U, Nimmo ER, Russell RK, Lofberg R, and Satsangi J (2006). Contribution of CARD15 variants in determining susceptibility to Crohn's disease in Sweden. *Scand J Gastroenterol* 41(6):700-5.

Torkvist L, Noble CL, Lordal M, Sjoqvist U, Lindforss U, Nimmo ER, Lofberg R, Russell RK, and Satsangi J (2007). Contribution of the IBD5 locus to Crohn's disease in the Swedish population. *Scand J Gastroenterol* 42(2):200-6.

Torok HP, Glas J, Tonenchi L, Mussack T, and Folwaczny C (2004). Polymorphisms of the lipopolysaccharide-signaling complex in inflammatory bowel disease: association of a mutation in the Toll-like receptor 4 gene with ulcerative colitis. *Clin Immunol* 112(1):85-91.

Torok HP, Glas J, Tonenchi L, Lohse P, Muller-Myhsok B, Limbersky O, Neugebauer C, Schnitzler F, Seiderer J, Tillack C, Brand S, Brunnler G, Jagiello P, Epplen JT, Griga T, Klein W, Schiemann U, Folwaczny M, Ochsenkuhn T, and Folwaczny C (2005). Polymorphisms in the DLG5 and OCTN cation transporter genes in Crohn's disease. *Gut* 54(10):1421-7.

Trachtenberg EA, Yang H, Hayes E, Vinson M, Lin C, Targan SR, Tyan D, Erlich H, and Rotter JI (2000). HLA class II haplotype associations with inflammatory bowel disease in Jewish (Ashkenazi) and non-Jewish Caucasian populations. *Human Immunology* 61(3):326-333.

Tremelling M, Waller S, Bredin F, Greenfield S, and Parkes M (2006). Genetic variants in TNF-alpha but not DLG5 are associated with inflammatory bowel disease in a large United Kingdom cohort. *Inflamm Bowel Dis* 12(3):178-84.

Tremelling M, Cummings F, Fisher SA, Mansfield J, Gwilliam R, Keniry A, Nimmo ER, Drummond H, Onnie CM, Prescott NJ, Sanderson J, Bredin F, Berzuini C, Forbes A, Lewis CM, Cardon L, Deloukas P, Jewell D, Mathew CG, Parkes M, and Satsangi J (2007). IL23R variation determines susceptibility but not disease phenotype in inflammatory bowel disease. *Gastroenterology* 132(5):1657-64.

Tromp G, Kuivaniemi H, Raphael S, Ala-Kokko L, Christiano A, Considine E, Dhulipala R, Hyland J, Jokinen A, Kivirikko S, Korn R, Madhatheri S, McCarron S, Pulkkinen L, Punnett H, Shimoya K, Spotila L, Tate A, and Williams CJ (1996). Genetic linkage of familial granulomatous inflammatory arthritis, skin rash, and uveitis to chromosome 16. *Am J Hum Genet* 59(5):1097-107.

Truelove SC, and Witts LJ (1955). Cortisone in ulcerative colitis; final report on a therapeutic trial. *Br Med J* 2(4947):1041-8.

Tukel T, Shalata A, Present D, Rachmilewitz D, Mayer L, Grant D, Risch N, and Desnick RJ (2004). Crohn disease: frequency and nature of CARD15 mutations in Ashkenazi and Sephardi/Oriental Jewish families. *Am J Hum Genet* 74(4):623-36.

Turunen P, Kolho KL, Auvinen A, Iltanen S, Huhtala H, and Ashorn M (2006). Incidence of inflammatory bowel disease in Finnish children, 1987-2003. *Inflamm Bowel Dis* 12(8):677-83.

Tysk C, Lindberg E, Jarnerot G, and Floderus-Myrhed B (1988). Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. *Gut* 29(7):990-6.

Uehara A, Fujimoto Y, Fukase K, and Takada H (2007). Various human epithelial cells express functional Toll-like receptors, NOD1 and NOD2 to produce anti-microbial peptides, but not proinflammatory cytokines. *Mol Immunol* 44(12):3100-11.

Urcelay E, Mendoza JL, Martinez A, Fernandez L, Taxonera C, Diaz-Rubio M, and de la Concha EG (2005). IBD5 polymorphisms in inflammatory bowel disease: association with response to infliximab. *World J Gastroenterol* 11(8):1187-92.

Urcelay E, Mendoza JL, Martin MC, Mas A, Martinez A, Taxonera C, Fernandez-Arquero M, Diaz-Rubio M, and de la Concha EG (2006). MDR1 gene: susceptibility in Spanish Crohn's disease and ulcerative colitis patients. *Inflamm Bowel Dis* 12(1):33-7.

Uthoff SM, Crawford NP, Eichenberger MR, Hamilton CJ, Petras RE, Martin ER, and Galandiuk S (2002). Association of ulcerative colitis with the inflammatory bowel disease susceptibility locus IBD2 in non-Jewish Caucasians and evidence of genetic heterogeneity among racial and ethnic populations with Crohn disease. *Am J Med Genet* 113(3):242-9.

Uyar FA, Saruhan-Direskeneli G, and Gul A (2004). Common Crohn's disease-predisposing variants of the CARD15/NOD2 gene are not associated with Behcet's disease in Turkey. *Clin Exp Rheumatol* 22(4 Suppl 34):S50-2.

Waller S, Tremelling M, Bredin F, Godfrey L, Howson J, and Parkes M (2006). Evidence for association of OCTN genes and IBD5 with ulcerative colitis. *Gut* 55(6):809-14.

van der Linde K, Boor PP, Houwing-Duistermaat JJ, Crusius BJ, Wilson PJ, Kuipers EJ, and de Rooij FW (2007). CARD15 mutations in Dutch familial and sporadic inflammatory bowel disease and an overview of European studies. *Eur J Gastroenterol Hepatol* 19(6):449-59.

van Duist MM, Albrecht M, Podswiadek M, Giachino D, Lengauer T, Punzi L, and De Marchi M (2005). A new CARD15 mutation in Blau syndrome. *Eur J Hum Genet* 13(6):742-7.

van Heel DA, Carey AH, and Jewell DP (2001). Identification of novel polymorphisms in the beta7 integrin gene: family-based association studies in inflammatory bowel disease. *Genes Immun* 2(8):455-60.

van Heel DA, Fisher SA, Kirby A, Daly MJ, Rioux JD, and Lewis CM (2004). Inflammatory bowel disease susceptibility loci defined by genome scan meta-analysis of 1952 affected relative pairs. ***Hum. Mol. Genet.*** 13(7):763-770.

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, and Playford RJ (2005a). Muramyl dipeptide and toll-like receptor sensitivity in NOD2-associated Crohn's disease. *Lancet* 365(9473):1794-6.

van Heel DA, Ghosh S, Hunt KA, Mathew CG, Forbes A, Jewell DP, and Playford RJ (2005b). Synergy between TLR9 and NOD2 innate immune responses is lost in genetic Crohn's disease. *Gut* 54(11):1553-7.

van Heel DA, Hunt KA, King K, Ghosh S, Gabe SM, Mathew CG, Forbes A, and Playford RJ (2006). Detection of muramyl dipeptide-sensing pathway defects in patients with Crohn's disease. *Inflamm Bowel Dis* 12(7):598-605.

Van Limbergen J, Russell RK, Nimmo ER, Drummond HE, Smith L, Davies G, Anderson NH, Gillett PM, McGrogan P, Hassan K, Weaver L, Bisset WM, Mahdi G, Wilson DC, and Satsangi J (2007a). IL23R Arg381Gln is associated with childhood onset inflammatory bowel disease in Scotland. *Gut* 56(8):1173-4.

Van Limbergen J, Russell RK, Nimmo ER, Ho GT, Arnott ID, Wilson DC, and Satsangi J (2007b). Genetics of the innate immune response in inflammatory bowel disease. *Inflamm Bowel Dis* 13(3):338-55.

Van Limbergen J, Russell RK, Nimmo ER, Drummond HE, Smith L, Anderson NH, Davies G, Gillett PM, McGrogan P, Weaver LT, Bisset WM, Mahdi G, Arnott ID, Wilson DC, and Satsangi J (2008). Autophagy gene ATG16L1 influences susceptibility and disease location but not childhood-onset in Crohn's disease in Northern Europe. *Inflamm Bowel Dis* 14(3):338-46.

Waschke KA, Villani AC, Vermeire S, Dufresne L, Chen TC, Bitton A, Cohen A, Thomson AB, and Wild GE (2005). Tumor necrosis factor receptor gene polymorphisms in Crohn's disease: association with clinical phenotypes. *Am J Gastroenterol* 100(5):1126-33.

Watanabe T, Kitani A, Murray PJ, and Strober W (2004). NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. *Nat Immunol* 5(8):800-8.

Vavricka SR, Musch MW, Chang JE, Nakagawa Y, Phanvijhitsiri K, Waypa TS, Merlin D, Schneewind O, and Chang EB (2004). hPepT1 transports muramyl dipeptide, activating NF-kappaB and stimulating IL-8 secretion in human colonic Caco2/bbe cells. *Gastroenterology* 127(5):1401-9.

Weersma RK, Zhernakova A, Nolte IM, Lefebvre C, Rioux JD, Mulder F, van Dullemen HM, Kleibeuker JH, Wijmenga C, and Dijkstra G (2008). ATG16L1 and IL23R Are Associated With Inflammatory Bowel Diseases but Not With Celiac Disease in The Netherlands. *Am J Gastroenterol* 103(3):621-7.

Wehkamp J, Harder J, Weichenthal M, Schwab M, Schaffeler E, Schlee M, Herrlinger KR, Stallmach A, Noack F, Fritz P, Schroder JM, Bevins CL, Fellermann K, and Stange EF (2004). NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. *Gut* 53(11):1658-64.

Wehkamp J, Salzman NH, Porter E, Nuding S, Weichenthal M, Petras RE, Shen B, Schaeffeler E, Schwab M, Linzmeier R, Feathers RW, Chu H, Lima H, Jr., Fellermann K, Ganz T, Stange EF, and Bevins CL (2005). Reduced Paneth cell alpha-defensins in ileal Crohn's disease. *Proc Natl Acad Sci U S A* 102(50):18129-34.

Vermeire S, Louis E, Rutgeerts P, De Vos M, Van Gossum A, Belaiche J, Pescatore P, Fiasse R, Pelckmans P, Vlietinck R, Merlin F, Zouali H, Thomas G, Colombel JF, and Hugot JP (2002a). NOD2/CARD15 does not influence response to infliximab in Crohn's disease. *Gastroenterology* 123(1):106-11.

Vermeire S, Wild G, Kocher K, Cousineau J, Dufresne L, Bitton A, Langelier D, Pare P, Lapointe G, Cohen A, Daly MJ, and Rioux JD (2002b). CARD15 genetic variation in a Quebec population: prevalence, genotype-phenotype relationship, and haplotype structure. *Am J Hum Genet* 71(1):74-83.

Vermeire S, Rutgeerts P, Van Steen K, Joossens S, Claessens G, Pierik M, Peeters M, and Vlietinck R (2004). Genome wide scan in a Flemish inflammatory bowel disease population: support for the IBD4 locus, population heterogeneity, and epistasis. *Gut* 53(7):980-6.

Vermeire S, Pierik M, Hlavaty T, Claessens G, van Schuerbeeck N, Joossens S, Ferrante M, Henckaerts L, Bueno de Mesquita M, Vlietinck R, and Rutgeerts P (2005). Association of organic cation transporter risk haplotype with perianal penetrating Crohn's disease but not with susceptibility to IBD. *Gastroenterology* 129(6):1845-53.

Vermeire S, and Rutgeerts P (2005). Current status of genetics research in inflammatory bowel disease. *Genes Immun* 6(8):637-45.

Vermeire S, Van Assche G, and Rutgeerts P (2006). Laboratory markers in IBD: useful, magic, or unnecessary toys? *Gut* 55(3):426-31.

Vignal C, Singer E, Peyrin-Biroulet L, Desreumaux P, and Chamaillard M (2007). How NOD2 mutations predispose to Crohn's disease? *Microbes Infect* 9(5):658-63.

Williams CN, Kocher K, Lander ES, Daly MJ, and Rioux JD (2002). Using a genome-wide scan and meta-analysis to identify a novel IBD locus and confirm previously identified IBD loci. *Inflamm Bowel Dis* 8(6):375-81.

Vind I, Vieira A, Hougs L, Tavares L, Riis L, Andersen PS, Locht H, Freitas J, Monteiro E, Christensen IJ, and Munkholm P (2005). NOD2/CARD15 gene polymorphisms in Crohn's disease: a genotype-phenotype analysis in Danish and Portuguese patients and controls. *Digestion* 72(2-3):156-63.

Vind I, Riis L, Jess T, Knudsen E, Pedersen N, Elkjaer M, Bak Andersen I, Wewer V, Norregaard P, Moesgaard F, Bendtsen F, and Munkholm P (2006). Increasing incidences of inflammatory bowel disease and decreasing surgery rates in Copenhagen City and County, 2003-2005: a population-based study from the Danish Crohn colitis database. *Am J Gastroenterol* 101(6):1274-82.

Windheim M, Lang C, Peggie M, Plater LA, and Cohen P (2007). Molecular mechanisms involved in the regulation of cytokine production by muramyl dipeptide. *Biochem J* 404(2):179-90.

Wu H, and Arron JR (2003). TRAF6, a molecular bridge spanning adaptive immunity, innate immunity and osteoimmunology. *Bioessays* 25(11):1096-105.

Xia B, Crusius JB, Wu J, Zwiers A, van Bodegraven AA, and Pena AS (2003). Signal transducer and activator of transcription 6 gene G2964A polymorphism and inflammatory bowel disease. *Clin Exp Immunol* 131(3):446-50.

Yamamoto-Furusho JK, Uscanga LF, Vargas-Alarcon G, Ruiz-Morales JA, Higuera L, Cutino T, Rodriguez-Perez JM, Villarreal-Garza C, and Granados J (2003). Clinical and genetic heterogeneity in Mexican patients with ulcerative colitis. *Hum Immunol* 64(1):119-23.

Yamamoto-Furusho JK, and Podolsky DK (2007). Innate immunity in inflammatory bowel disease. *World J Gastroenterol* 13(42):5577-80.

Yamazaki K, Takazoe M, Tanaka T, Kazumori T, and Nakamura Y (2002). Absence of mutation in the NOD2/CARD15 gene among 483 Japanese patients with Crohn's disease. *J Hum Genet* 47(9):469-72.

Yamazaki K, Takazoe M, Tanaka T, Ichimori T, Saito S, Iida A, Onouchi Y, Hata A, and Nakamura Y (2004). Association analysis of SLC22A4, SLC22A5 and DLG5 in Japanese patients with Crohn disease. *J Hum Genet* 49(12):664-8.

Yamazaki K, McGovern D, Ragoussis J, Paolucci M, Butler H, Jewell D, Cardon L, Takazoe M, Tanaka T, Ichimori T, Saito S, Sekine A, Iida A, Takahashi A, Tsunoda T, Lathrop M, and Nakamura Y (2005). Single nucleotide polymorphisms in TNFSF15 confer susceptibility to Crohn's disease. *Hum Mol Genet* 14(22):3499-506.

Yamazaki K, Onouchi Y, Takazoe M, Kubo M, Nakamura Y, and Hata A (2007). Association analysis of genetic variants in IL23R, ATG16L1 and 5p13.1 loci with Crohn's disease in Japanese patients. *J Hum Genet* 52(7):575-83.

Yang H, McElree C, Roth MP, Shanahan F, Targan SR, and Rotter JI (1993). Familial empirical risks for inflammatory bowel disease: differences between Jews and non-Jews. *Gut* 34(4):517-24.

Yang H, Ohmen JD, Ma Y, Bentley LG, Targan SR, Fischel-Ghodsian N, and Rotter JI (1999a). Additional evidence of linkage between Crohn's disease and a putative locus on chromosome 12. *Genet Med* 1(5):194-8.

Yang H, Plevy SE, Taylor K, Tyan D, Fischel-Ghodsian N, McElree C, Targan SR, and Rotter JI (1999b). Linkage of Crohn's disease to the major histocompatibility complex region is detected by multiple non-parametric analyses. *Gut* 44(4):519-526.

Yap LM, Ahmad T, and Jewell DP (2004). The contribution of HLA genes to IBD susceptibility and phenotype. *Best Pract Res Clin Gastroenterol* 18(3):577-96.

Ye H, Arron JR, Lamothe B, Cirilli M, Kobayashi T, Shevde NK, Segal D, Dzivenu OK, Vologodskaia M, Yim M, Du K, Singh S, Pike JW, Darnay BG, Choi Y, and Wu H (2002). Distinct molecular mechanism for initiating TRAF6 signalling. *Nature* 418(6896):443-7.

Zaahl MG, Winter T, Warnich L, and Kotze MJ (2005). Analysis of the three common mutations in the CARD15 gene (R702W, G908R and 1007fs) in South African colored patients with inflammatory bowel disease. *Mol Cell Probes* 19(4):278-81.

Zhou Z, Lin X-y, Akolkar PN, Gulwani-Akolkar B, Levine J, Katz S, and Silver J (2002). Variation at NOD2/CARD15 in familial and sporadic cases of Crohn's disease in the Ashkenazi Jewish population. *The American Journal of Gastroenterology* 97(12):3095-3101.

Zouiten-Mekki L, Zaouali H, Boubaker J, Karoui S, Fekih M, Matri S, Hamzaoui S, Filali A, Chaabouni H, and Hugot JP (2005). CARD15/NOD2 in a Tunisian population with Crohn's disease. *Dig Dis Sci* 50(1):130-5.