Role of ATG16L, NOD2 and IL23R in Crohn's disease pathogenesis

Saleh A. Naser
University of Central Florida

Melissa Arce
University of Central Florida

Anam Khaja
University of Central Florida

Marlene Fernandez
University of Central Florida

Najih Naser

See next page for additional authors
Find similar works at: https://stars.library.ucf.edu/facultybib2010
University of Central Florida Libraries http://library.ucf.edu

This Review is brought to you for free and open access by the Faculty Bibliography at STARS. It has been accepted for inclusion in Faculty Bibliography 2010s by an authorized administrator of STARS. For more information, please contact STARS@ucf.edu.

Recommended Citation
Naser, Saleh A.; Arce, Melissa; Khaja, Anam; Fernandez, Marlene; Naser, Najih; Elwasila, Sammer; and Thanigachalam, Saisathya, "Role of ATG16L, NOD2 and IL23R in Crohn's disease pathogenesis" (2012). Faculty Bibliography 2010s. 3069.
https://stars.library.ucf.edu/facultybib2010/3069
Authors
Saleh A. Naser, Melissa Arce, Anam Khaja, Marlene Fernandez, Najih Naser, Sammer Elwasila, and Saisathya Thanigachalam

This review is available at STARS: https://stars.library.ucf.edu/facultybib2010/3069
Role of ATG16L, NOD2 and IL23R in Crohn’s disease pathogenesis

Saleh A Naser, Melissa Arce, Anam Khaja, Marlene Fernandez, Najih Naser, Sammer Elwasila, Saisathya Thanigachalam

Abstract

Inflammatory bowel disease is a group of diseases that includes Crohn's disease (CD) and ulcerative colitis. CD is characterized as a chronic inflammatory disease of the gastrointestinal tract, ranging from the mouth to the anus. Although there are gross pathological and histological similarities between CD and Johne's disease of cattle, the cause of CD remains controversial. It is vital to understand fully the cause of this disease because it affects approximately 500,000 people in North America and Europe. It ranges from 27 to 48 cases per 100,000 people. There are many theories on the cause of CD ranging from possible association with environmental factors including microorganisms to imbalance in the intestinal normal flora of the patients. Regardless of the environmental trigger, there is strong evidence that a genetic disposition is a major key in acquiring CD. Many studies have proven the link between mutations in the ATG16L, NOD2/CARD15, IBD5, CTLA4, TNFSF15 and IL23R genes, and CD. The purpose of this review is to examine all genetic aspects and theories of CD, including up to date multiple population studies performed worldwide.

INTRODUCTION

The first description of Crohn’s disease (CD) was made in 1769 by an Italian physician, Giovanni Battista Morgagni, when he diagnosed a man with chronic diarrhea. In 1898, consecutive cases were reported by John Berg, and then in 1904 by Antoni Lesniowski. Throughout the 1920s and 1930s, young adults were thought to have this same condition as they suffered from symptoms, such as abdominal cramps, diarrhea, fever and significant weight loss. In 1923, surgeons at Mt. Sinai Hospital in New York had also identified patients with comparable symptoms. In addition, in 1930, Dr. Burrill B Crohn saw a connection between this unknown debilitating condition and two of his patients. Consequently, Dr. Crohn and his
colleagues presented a paper in 1932, “Regional ileitis: a pathologic and chronic entity”, describing the features of this disease to the American Medical Association. CD was named after Burrill Crohn and it became an official medical entity in 1932.

CD is a chronic inflammatory disease that can affect any portion of the digestive tract including the mouth, esophagus, and small and large intestines, but is most common in the ileum. It is also characterized as an autoimmune disease in which the body attacks itself and causes inflammation. In its mild form, it causes erosions called aphthous ulcers in the inner surface of the bowel. In severe cases, deeper and larger ulcers develop that can lead to bowel obstruction and holes in the bowel wall. If a hole in the bowel wall arises, infection to neighboring organs can occur. CD branches off to many forms depending on the location of erosion. If these symptoms take place within the large intestine, it is called Crohn’s or granulomatous colitis. If it occurs in the small intestine, it is known as Crohn’s enteritis, and more specifically if it occurs in the ileum, it is called Crohn’s ileitis. During severe cases in which the small and large intestine are both involved, it is known as Crohn’s enterocolitis or ileocolitis. CD is separated into three phenotypes: non-stricturing and non-penetrating, stricturing and penetrating and penetrating CD.

CD is most prevalent in North America and Europe, and least prevalent among African Americans and Asians. It affects approximately 500,000 people in North America and Europe. It ranges from 27 to 48 cases per 100,000 people. There is no difference in prevalence among males or females. Individuals with siblings affected by CD have a higher risk of acquiring the disease. There is evidence of a cause from environmental factors, thus, there are a higher number of cases in western industrialized countries. Symptoms of CD typically begin in the teens and twenties and then go into remission on and off with appropriate therapies. There is a peak incidence between 50 years and 70 years of age, which often leads to major complications due to age and the necessity of surgery.

Although the etiology is still unknown, there are many theories about what causes CD and ulcerative colitis (UC). Many believe it is caused by environmental factors, such as certain foods, bacteria, viruses, or cigarette smoke, which all can trigger an immune system response. Scientists have linked inflammatory bowel diseases (IBDs) as an autoimmune problem. In a healthy person, the immune system defends the body against harmful microbes that have entered it. Upon triggering the immune system, an inflammatory response occurs in which immune cells aggregate at the site of infection and overcome the threat. There are microbes native to our bodies that are useful rather than harmful to which the immune system does not trigger a response. In patients with CD, the immune system will attack these native luminal bacteria, disrupting the normal flora, thus characterizing this condition as an autoimmune disease.

Recent research has indicated specific genetic variations as a direct cause of CD and UC. The genetic aspects of CD have been linked by observing familial clustering of IBD cases. Genetic variations in the ATG16L1, NOD2/CARD15 and IL23R genes have strongly been linked to the onset of CD. Not only are individual gene mutations listed as a cause of CD, but a combination of them has also been shown in CD patients by conducting many population studies. There have also been many studies that have predicted surgical outcomes in both adults and children with specific genetic variations.

Diagnosis of CD can be tricky and requires a number of tests to be certain. Colonoscopy is the most effective way to diagnose CD but not in all cases because it only allows the physician to visualize the colon, ileum, and lower portion of the small intestine. If the ulcers are located within the upper portion of the small intestine, this test will not be effective. In this case, a barium follow-through X-ray is useful because barium sulfate gives fluoroscopic images of the bowel and the physician can see areas of inflammation and narrowing. Another effective method of diagnosis is the use of white blood cell scans. During this procedure, white blood cells are tagged with a radioisotope and then injected back into the patient. At specific intervals of time, the scan can locate accumulations of white blood cells in the intestine at the site of CD. This method is also useful to monitor the disease and show effectiveness in other therapies. Furthermore, a simple blood test can also diagnose CD because it can determine whether the patient is anemic or has a vitamin B12 deficiency because vitamin B12 is absorbed in the ileum and a deficiency can be due to ileitis.

CD causes a wide variety of symptoms and can be confused with UC, which is a similar disease, both under the group of IBDs. UC is only found in the colon and affects the mucosal membrane, whereas CD can occur anywhere throughout the gastrointestinal (GI) tract and affects the thickness of the GI wall. Individuals with CD can experience flare-ups followed by remissions. It can vary from one flare-up in a lifetime to multiple flare-ups that need surgical treatment. Symptoms include persistent diarrhea, abdominal pain in the affected area, fever, and weight loss. There are also signs and symptoms that may occur unrelated to the GI tract, such as reddening and inflammation of the eye, joint pain, skin lesions, and sores inside the mouth.

Currently, there is no cure for CD. Treatment is focused on relieving the symptoms and putting it into remission. Since CD is characterized as an autoimmune disease, medications to suppress the immune system include 5-aminosalicylic acid and steroids, such as prednisone. Antibiotics such as clarithromycin, ampicillin and metronidazole, can also be used. More than 50% of patients with CD will have to undergo surgical treatment to correct a fistula, drain an abscess, open a narrow or obstructed bowel, or remove a segment of infected intestine.
AUTOPHAGY-RELATED 16-LIKE 1 PROTEIN COMPLEX

Autophagy is a catabolic process of intracellular degradation in which cytoplasmic components are sequestered within vesicles and delivered to the lysosomes. Cells use this pathway during nutrient starvation because they can break down non-vital components and use them as nutrients. Autophagy plays a role during infection by helping rid the cell of foreign antigens by breakdown of the pathogen. This pathway can also be implemented as a repair mechanism to degrade damaged organelles and proteins. Autophagosomes, formed by the fusion of lysosomes and vesicles, are also implicated in the processing of intracellular bacteria. If the gene responsible for autophagy is mutated, it can cause a shift in normal flora as previously mentioned, and lead to many GI problems, which has been pointed out as a possible cause of CD. If the cell cannot regain nutrients or fight off foreign antigens within the GI tract, these cells will undergo programmed cell death and cause tissue damage. This damage can be seen as lesions and ulcers within the intestines, creating dead infected patches of tissue along the GI tract. The only option of treatment is surgery to remove the sections of the intestines with the diseased tissue so that the necrosis will not spread among neighboring cells.

An autophagosome, a double-membraned vesicle formed by autophagy, envelopes part of the cytoplasm and delivers it to the lysosomes where it is degraded and recycled. There have been approximately 30 autophagy-related (Atg) genes identified, with two proteins having ubiquitin-like characteristics, Atg12 and Atg8. These proteins covalently modify their target protein with molecules such as ubiquitin-like proteins to tag them for degradation. Both proteins also contain a conserved ubiquitin-fold region. Autophagosomes use two conjugation systems, the Atg12 and LC3-II systems. These systems were first discovered during yeast genetic studies revealing a set of 17 ATG genes involved in the autophagy pathway. In the Atg12 conjugation system, an Atg12-Atg5-Atg16L complex forms, and dissociates from the membrane just before or after completion of the autophagosome. The ATG16L1 protein is expressed in the colon, small intestine, intestinal epithelial cells, leukocytes, and spleen. Recent independent studies have shown that an ATG16L mutation, located on chromosome 2, is associated with the onset of ileal CD, and is therefore a key molecule in elucidating the genetic aspects of this disease.

Multiple studies have been performed with each resulting in the same conclusion that ATG16L is implicated in CD. During a genome-wide survey of 19 779 non-synonymous single nucleotide polymorphisms (SNPs), Thr300Ala within the N terminus of ATG16L was found to be highly associated with CD by using a haplotype and regression analysis. This study used a total of 735 CD patients, 368 controls and 72 SNPs. A second report from the North American CD genome-wide study submitted by Rioux and colleagues also shows an association with ATG16L using a case-control analysis in 988 CD patients and 1007 controls. Another group of German and British collaborators demonstrated that rs2241880, another non-synonymous variant of the ATG16L gene on chromosome 2q37.1, is implicated in the autophagy pathway.

In a study performed on an Italian cohort, the same polymorphism, rs2241880, was observed in 667 CD and 668 UC patients. Both the frequency of the G allele and number of carriers of the G allele were increased in CD patients when compared to the controls. These differences were only significant in the adult subgroup, which could be due to the small sample size of the pediatric subgroup. In comparison, there were no significant allele or genotype frequencies found between UC patients and controls for the groups as a whole. During analysis of genotype/phenotype correlation of the rs2241880 SNP, there were no associations with disease location, behavior, and age at diagnosis based on the Montreal Classification of CD. There were also no associations found in sex, smoking, and perianal fistulae. For the rs2241880 variant, recent studies, specifically by Prescott, demonstrate an association with the ileal form of CD with or without colonic involvement, but not with isolated colonic disease.

In addition, a study from Oxford compared 645 CD patients with 1190 controls and showed an association of ATG16L1 with CD. To understand fully the function of this gene, a study utilized oligo-based silencing RNA directed against ATG16L1 isoforms, where autophagy was induced by Salmonella typhimurium in ATG16L1 knockdown HEK293 cells. There was a significant difference between the knockdown cells compared to the control cells during the autophagy pathway. It is clear to say that variants of this gene have been proven without a doubt to be directly associated with CD because autophagy plays a critical role in disease pathogenesis. Further research needs to focus on understanding how ATG16L1 variants contribute to disease susceptibility in IBD patients, and their possible therapeutic implications.

TUMOR NECROSIS FACTOR SUPERFAMILY 15

Tumor necrosis factor superfamily 15 (TNFSF15) is a Th-1 polarizing cytokine involved in systemic inflammation. TNF functions to regulate immune cells, induce apoptosis, induce inflammation, and inhibit tumorigenesis. TNFs are produced by macrophages, lymphoid cells, mast cells and endothelial cells. During immunological studies, it has been found that CD patients have an increased expression of TNFSF by multiple cells in the intestinal tissues when compared to controls. The TNFSF15 gene is a candidate for increased susceptibility in IBD. It binds to a specific T-cell receptor to enhance...
cytokine-induced interferon expression in mucosal CD4+ T cells[1].

In 2005, the first genome study involving IBD tested nearly 80,000 SNPs in Japanese CD patients. This study identified haplotypes within the TNF5F15 gene, which included seven SNPs within a 280-kb region on chromosome 9q32[20]. By resequencing TNFSF15 from the same CD cases but with a new control group, TNFSF15 was found to be strongly associated with CD with an odds ratio (OR) of 2.17 (95% CI: 1.78-2.66), $P = 1.71 \times 10^{-14}$[15]. The Japanese wanted to see if the same patterns were seen in other population groups so they replicated their associate in two panels from Oxford, United Kingdom. Although the risk haplotype was identified in both cohorts, there was a weaker effect size ($P = 0.02$ in both family-based and case-control association panels). In addition, another study involving a Jewish cohort also showed an association of TNFSF15 with CD, and also suggested that in response to Fc-gamma receptor stimulation, TNF5F15 gene variation aggravates induction of TNFSF15[14]. However, in a separate study using a Belgian CD cohort, no significant association was observed between CD and TNFSF15[6]. This may have been due to different marker genotypes or differences in susceptibility genes between Asian and European cohorts[6]. Not enough studies have been performed regarding TNFSF15 and its possible implications in CD. Future studies have to focus on different populations to provide efficient insight.

**NOD2/CARD15 GENE**

Nucleotide-binding oligomerization domain containing 2 (NOD2), located on chromosome 16q12, is a protein that plays an essential role in the immune system by controlling commensal bacterial flora in the intestine[17]. NOD2 belongs to a nucleotide-binding domain, leucine-rich repeat family of cytoplasmic proteins that may detect a variety of bacteria by acting as an intracellular sensor for bacterial peptidoglycan[18]. It has the ability to respond to N-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) via the leucine-rich repeat domain. The MDP is conserved in both Gram-negative and positive bacteria[10,19]. This leucine-rich repeat domain plays a role in protein-protein interactions and the middle portion of the protein is responsible for self-oligomerization. The N-terminal portion of NOD2 contains two caspase recruitment domains (CARDs) which play a role in apoptosis. The CARD15 gene, which encodes for the CARD domain within NOD2, has been specifically identified as a genetic factor for CD. Three SNPs were found to be independently associated with CD: rs2066844, rs2066845, and an insertion mutation 3020insC[21]. Each variant may result in distinct phenotypic expression of CD[4]. To activate NOD2, Rip2 kinase is required because it is necessary for downstream signaling of NOD2 and signaling cascades such as nuclear factor (NF)-κB and mitogen-activated protein kinase cascades[22].

The intestinal mucosa is constantly exposed to a large number of commensal microorganisms; the majority of which inhabit the large intestine. In a healthy individual, there is a basal immune response elicited from the interaction between the intestinal immune system and commensal bacteria. This immune response is constantly present to protect the host from pathogenic and non-pathogenic bacteria. When there are changes within this balance, the intestines are susceptible to chronic intestinal inflammatory conditions, such as CD[23]. Multiple genetic studies have linked NOD2 with susceptibility to CD. However, NOD2-deficient mice do not develop colitis, suggesting that dysregulation of the NOD2 pathway is not sufficient to provoke CD[20]. This is not a surprise because the pathogenesis of CD is caused by several factors including environmental, dysfunctional immune system, and a shift in normal bacterial flora[21].

NOD2 is expressed in Paneth cells, which are found in the intestines. The exact functions of Paneth cells are still unknown, but they are likely to contribute to the host defense by secreting antibacterial compounds due to the presence of lysozyme. A NOD2 mutation can alter the function of Paneth cells, which alters their antimicrobial activity and leads to the development of ileal lesions, which correspond to the location of these cells. Of the three NOD2 mutations associated with CD, both rs2066844 and rs2066845 are the result of a two-amino-acid substitution in which rs2066844 is encoded by exon 4 and rs2066845 is encoded by exon 8[23]. The variant 1007fs is created by a frameshift mutation in exon 11[25]. Each of the three mutations occurs within or near the leucine-rich repeat and decrease the cells ability to activate NF-κB in response to peptidoglycan[8]. Interestingly, these mutations are observed in Caucasian patients but not in Japanese, Chinese and Korean patients with IBD, and they are very rare in African Americans with IBD[21,26]. An individual heterozygous for at least one NOD2 mutation is at a 2.4-fold increased risk of developing CD, whereas a homozygous individual for at least one NOD2 mutation is at a 20-40-fold increased risk when compared to healthy individuals[24].

Studies of each individual mutation in NOD2 have shown that 1007fs causes a decrease in defensin expression[26]. Defensins are cells of the immune system that assist in killing phagocytized bacteria. They function by binding to the microbial cell membrane and forming a pore in the membrane that allows outward flow of nutrients and essential ions. CD patients who are homozygous and/or heterozygous for NOD2 mutations typically have lower defensin levels in their ileostomy fluid[27]. As a result of a mutation in NOD2, the function of this protein is diminished, therefore allowing subsequent entry of bacteria into epithelial cells because they are no longer able to recognize them. This in turn alters the bacterial population in the intestines and thus, defensins are not able to function correctly because they are working in an impaired bactericidal capacity[22].

In a recent study by Van Limbergen et al[7], it was
proven that NOD2 is required for the regulation of commensal microbiota in the intestine. The regulation of NOD2 depends on the downstream kinase Rip2 because Rip2-deficient mice fail to establish and regulate commensal bacteria in their terminal ileum. NOD2-deficient mice do not develop spontaneous intestinal inflammation. In conclusion, the NOD2–Rip2 pathway is critical for the regulation of homeostasis between the body’s normal bacterial flora and innate immunity. As a controlled balance, it has been found that the expression of NOD2 and Rip2 is dependent upon and regulated by the presence of commensal bacteria positively regulate NOD2, and in turn negatively regulate the normal flora. NOD2 mutations directly affect the ileum in CD patients, thus, it is possible that they are responsible for the composition of the bacterial flora in the terminal ileum. This may facilitate both disease pathology and progression.

Although the exact mechanism by which NOD2 contributes to the control of commensals in the intestines is still not known, there are many possible theories proposed by this study. The first theory entertains the possibility that NOD2 regulates the commensal flora through the bactericidal activity of ileal crypt secretions. The second theory is that NOD2 regulates the adaptive immune system by inducing lymphoid tissue generation. The third theory describes how NOD2 expression in myeloid lineage cells contributes indirectly to maintain the normal microbiota flora. The fourth theory states that there may be other cells in the intestines, in addition to Paneth cells, that may play a role in the regulation of commensal bacteria in the intestines. Further research is needed to elucidate this mechanism.

It has been reported that both adults and pediatric cohorts share a strong association between the NOD2 variants and ileal disease location. Some studies have suggested that adult patients show a correlation in fibrostenotic behavior and NOD2 mutation status, whereas other studies have failed to replicate the same phenotypic effect. It is necessary to study different CD cohorts from different countries to obtain an accurate effect of each mutation. Exploring the genotype-phenotype interactions in children is beneficial because they show a higher gene dosage and have less environmental influences.

A study by Lacher and colleagues has explored the association of the NOD2 mutation in German pediatric CD patients and the risk of surgery. The risk of surgery in children is important to predict because surgery is sometimes the only method of treatment. Out of 171 young CD patients, 78 (45.6%) carried at least one NOD2 mutation, with 11 being compound heterozygous, and 14 being homozygous for two NOD2 mutations. The presence of rs2066844 was found in 29 (17%) children, rs2066845 was found in 18 (10.5%), and 1007fs was found in 42 (24.6%). Overall, one out of three German children with CD had at least one NOD2 mutation. In comparison, 36% of adult CD patients were heterozygous for at least one NOD2 mutation: 17.2% for rs2066844, 9.6% for rs2066845, and 11.7% for 1007fs. In conclusion, the genetic alterations were observed more predominantly in German pediatric CD patients than in adults. The study also looked into each mutation and its association with localization and symptoms of the disease. A 4.73-fold increased risk of isolated ileal localization was observed in patients that were identified as 1007fs carriers when compared to children with none of the NOD2 mutations. Although the 1007fs carriers were predisposed to ileal disease, there was no involvement of the ileocolonic or upper GI tract. The next characterization the study explored was the association of NOD2 mutations and stricturing disease or perianal fistulae in their German pediatric CD cohort. Only 17% of patients showed a stricturing phenotype and 18.1% had a perianal fistula. Among the children that showed a stricturing phenotype, 79.3% required surgery and those with the 1007fs mutation had surgical complications. The outcome showed a 9.8-fold increase in surgical complications when the child carried at least one allele for the 1007fs mutation. Not only is the 1007fs mutation strongly associated with isolated ileal disease, but children are at a high risk for surgical intervention. Therefore, this mutation can act as a prognostic tool in Caucasian children with CD.

Another study by Jurgens and colleagues has investigated the presence of fistulas and their association with NOD2 homozygosity and how they predict intestinal stenosis in CD patients. It was observed that patients with fistulas had simultaneous intestinal stenosis. In another study using the same research, it was found that NOD2/CARD15 variants, especially with 1007fs homozygosity, could predict the occurrence of intestinal stenosis. By using a strict screening process based on phenotypes including stenoses (stricturing CD) and fistulae (penetrating CD), and genotype, the study isolated a total of 145 patients. One hundred and twenty-five of the patients had penetrating CD with simultaneous stenosis within a 6-mo interval. It was also found that all 14 CD patients homozygous for NOD2 variants suffered from stenosis. To no one’s surprise, 11 out of the 14 patients with stenosis and fistulae carried the NOD2 1007fs mutation. These data confirm the strong risk factor of the 1007fs NOD2 mutation in the prevalence of intestinal stenosis, which may be related to the decreased intestinal barrier function found in CD patients with NOD2/CARD15 variant mutations. This study has isolated the 1007fs variant as the cause in disrupting the intestinal barrier and causing the many problems often observed in CD patients.

To investigate whether stenosis occurs in a specific anatomical region, 223 patients with 248 stenoses located at different intestinal regions were examined. The most common anatomical region was the terminal ileum with 68.8% of stenoses, followed by 11.7% found in the rectosigmoidal segment, and 9.3% in the jejunum or proximal ileum. In conclusion, homozygosity in the NOD2/
CARD15 mutation is a strong risk factor for intestinal stenosis\(^ {[20]}\). When the study looked into the possible association between stenosis and interleukin-23 receptor (IL-23R) mutation variants, there was a weak connection with no influence on stenosis\(^ {[20]}\). This study suggests classifying CD into four disease phenotypes instead of the current three in the Montreal Classification System. The four new classifications would be: non-stricturing\(^ {[23]}\), non-fistulizing CD\(^ {[23]}\); stricturing, non-fistulizing CD\(^ {[23]}\); non-stricturing, fistulizing CD\(^ {[23]}\); and stricturing, fistulizing CD\(^ {[23]}\). It is important to link any possible association between fistulas and stenoses because there is a strong risk factor for recurrence of CD after surgery\(^ {[20]}\).

Many studies have investigated North American, European and Asian countries and their CD patients with specific genotypes. Interestingly, the NOD2/CARD15 mutations are very rare and even absent in Asians (Japanese, Chinese and Korean), Arabs, Africans and African Americans\(^ {[26]}\). A study by Baptista and colleagues has concentrated on a South American population for the first time. There were a total of 187 CD patients used for the study with a median age of 33 years and a median age of onset of 23 years\(^ {[24]}\). Their patients were ethnically classified into the following groups: 58.8% were in the Brazilian subgroup, 36.9% shared a common European ancestry and were in the European-Brazilian subgroup, three were Amerindian-Brazilian, and two were Afro-Brazilian\(^ {[34]}\). The alleles related to CD within the CARD15, rs2066844 and 3020insC variants were significant for CD susceptibility\(^ {[24]}\). Both the rs2066845 and 1007fs variants failed to show any significant association\(^ {[24]}\). Among their patients, 30% had at least one NOD2/CARD15 variant allele\(^ {34}\). The frequency of rs2066844 (9.63%) in the Brazilian CD cohort was consistent with the reports for European populations\(^ {[24]}\). In conclusion, this study confirmed that CARD15 variants lead to greater susceptibility to CD in the Brazilian population\(^ {[24]}\).

**INTERLEUKIN-23 RECEPTOR**

IL-23R is a protein consisting of an IL-12β1 and an IL-23R chain\(^ {[27]}\). The molecular location of the IL-23R gene is on chromosome 1 and is formed by the binding of IL-12p40 and a p19 protein\(^ {[31]}\). It is highly expressed on the cell membrane of memory T cells and other immune cells, such as natural killer cells, monocytes, and dendritic cells, which identify foreign substances to defend the body against infection. It is highly involved in the mediation of proinflammatory activities by the production of IL-17 \textit{in vitro} the activation of Th17 lymphocytes\(^ {[30]}\). IL-23R interacts with IL-23, which is a cytokine that regulates the activity of immune cells and plays an important role in the inflammatory response against infection by bacteria and viruses. It also has been suggested that the functional IL-23R pathway polymorphisms play a role in modulating neonatal development of intestinal tolerance and bacterial colonization\(^ {[3]}\).

Th-17 lymphocytes are a distinct subset of T-helper cells, which mainly produce IL-17 and to a lesser extent IL-6 and TNF-α\(^ {[24]}\). IL-17 \textit{in vitro} and \textit{in vivo} acts as a potent inflammatory cytokine and is involved in the destruction of cartilage and bone, as seen in rheumatoid arthritis\(^ {[12]}\). It has been reported that IL-23 could be a key regulator in the differentiation of Th17 lymphocytes from memory T cells. It also has been suggested that IL-23 plays a role in providing a survival advantage to already differentiated Th17 cells\(^ {[29]}\). The expression of the heterodimeric receptor complex, IL-23R and IL-12Rβ1, regulates activities of IL-23\(^ {[24]}\). Therefore, the IL-23-IL17 cytokine axis is a key pathogenic mechanism that mediates the development and progress of inflammation by Th17 cells. The role for the IL23-IL17 axis in CD patients was supported in human patients and animal models of colitis\(^ {[30]}\). Both cytokines are increased in knockout mouse models of IBD. More specifically, IL-17 levels are increased in both intestinal mucosa and in the serum of CD patients\(^ {[32]}\). In a study using IL-17R knockout mouse models, an association was found with colitis and disease severity\(^ {[25]}\). Therefore, the use of anti-IL-12p40 antibody to treat CD patients is a therapeutic option because it is known to reduce production of IL-23 and IL-17 in the lamina propria cells\(^ {[12]}\). The mechanism of IL-23R is clearly important to understand because it is directly associated with CD.

During a genome-wide association study, 2877 DNA samples from IBD patients (two-thirds CD and one-third UC), identified rs11209026 as a possible protective variant, in the IL23 gene on chromosome 1p31\(^ {[14]}\). There are many other variants within IL23R that are associated with IBD, but rs11209026 has the strongest association with conferring protection against CD\(^ {[9]}\). Although the effect of IL23R variants is greatest in CD, it may have an overall effect on susceptibility to chronic intestinal inflammation.

Additional studies have confirmed the susceptibility of the IL23R gene to CD in North American and European populations. They include cohorts ranging from Scottish pediatric IBD, Belgian CD and an independent cohort of 883 families\(^ {[34]}\). Due to the role of IL-23 in activation of inflammatory responses, targeting this pathway may be a good therapeutic approach. Some promising research is underway using anti-p40, which blocks IL-23 and IL-12 activities. The variant allele, rs11209026, could be exploited to define clinical outcomes, such as a pharmacological approach to mimic the rs11209026 polymorphism\(^ {[9]}\).

A study by Schmechel and colleagues has shown a link in susceptibility to CD with Th17 cell function, IL-22 serum levels, and IL23R genotype. IL-22 is a strong activator of proinflammatory gene expression and upregulates SOC3 mRNA in intestinal epithelial cells\(^ {[29]}\). Recent evidence has shown that Th17 cells expressing IL23R play a key role in the mechanism by which IL23R modulates IBD susceptibility\(^ {[32]}\). Previous studies have shown that Th17 plays a role in autoimmune diseases,
such as rheumatoid arthritis and CD. However, Th17 is responsible for the important function of antimicrobial immunity at epithelial barriers where it produces cytokines such as IL-22. Because it produces IL-22 in epithelial barriers such as in the intestines, theoretically by testing for increased IL-22 serum levels, a physician can determine CD and disease activity.

It has been confirmed that IL-22 serum level is increased in CD and correlates with disease activity\cite{34}. IL-22 serum levels are also independent of CD phenotype and CARD15 genotype, but are modulated by IL23R polymorphisms\cite{35}. The study investigated IL-17 serum because Th17 cells also produce IL-17. There was no correlation between IL-22 and IL-17 serum levels\cite{35}. In total, serum levels of TNF-\alpha and IL-6 are used as inflammation markers in determining CD. There was no difference in TNF-\alpha and IL-6 levels whether CD was active or in remission, whereas IL-22 levels were significantly higher in active CD compared to CD in remission\cite{35}. Therefore, measuring IL-22 levels are clinically relevant in determining the disease activity in CD patients\cite{35}.

There are also strong associations between IL-22 serum levels and IL23R gene variants. Previous studies have shown both protective and inducing variants of IL23R in susceptibility to CD. Although rs1004819 is the CD increasing variant of IL23R, rs11209026 has shown to be a protective IL23R variant against CD. As predicted, when the IL-22 mean serum levels were tested against each variant, the serum levels were highest among SNPs that increased CD risk as opposed to the identified protective SNPs of IL23R, which had low serum levels\cite{35}. In contrast, the three main variants within NOD2/CARD15 did not show any differences in IL-22 serum levels\cite{35}. Although, the exact function of IL-22 in human IBD is still unknown, recent studies have observed increased \beta-defensin-2 expression and intestinal epithelial cell migration and proliferation upon stimulation with IL-22\cite{36}. These data ultimately suggests a clinically useful marker to assess disease severity and Th17 cell activity in CD patients\cite{35}.

As mentioned above, a rare glutamine allele, rs11209026, in IL23R conferred protection against CD during a recent genome-wide association study. Recent studies have proven that this variant protects against CD in both Jewish and non-Jewish populations. In addition, in a study involving IBD (both UC and CD) patients of Spanish Caucasian origin, the rs11209026 variant was seen to be most significantly associated with IBD protection (OR: 0.4; 95% CI: 0.3-0.7)\cite{36}. A study by Dubinsky and colleagues has investigated this rare allele further and how it protects pediatric CD patients. They used the transmission disequilibrium test (TDT) analysis and genotyping of whole blood samples from children with IBD and their two parents. This rare rs11209026 SNP was present in 2.67% non-Jewish CD patients and 2.94% of non-Jewish UC patients\cite{36}. The TDT demonstrated that the allele was under-transmitted in all CD offspring and confirmed the negative association between rs11209026 and CD\cite{35}.

Another way to confirm the association of alleles with CD and UC is to use a population that is genetically isolated, such as the Finnish population. This will provide an advantage in molecular genetic studies in complex disorders\cite{36}. A study by Lappalainen has used such a strategy to confirm the association of IL23R, TNFRSF1A, and the HLA-DRB1*0103 allele variants. The strongest association of IL23R with the marker rs2201841 (IL-23R risk variant) showed a frequency of 37.2% in CD. In most studies, the non-synonymous SNP, rs11209026 (the protective IL23R allele), has had the highest association, but is only marginally associated with Finnish CD\cite{35}. No association has been observed between the IL23R markers and UC patients\cite{35}.

Previous studies have shown that the HLA-DRB1*0103 allele is associated with both UC and CD. In the Finnish population, this allele only has a frequency of 0.6%, which is not statistically significant, whereas it is significant for UC and IBD\cite{36}. When looking into the genotype-phenotype association, patients carrying the rare HLA-DRB1*0103 allele have colonic involvement in CD\cite{36}. During the TNFRSF1A analysis, the investigators genotyped a rare A36G variant and an IVS6+10A (rs1800693) variant. CD patients with both variants often show ileocolonic disease in comparison with patients without these two variants\cite{36}. The weak association is probably due to the small sample size. Interestingly, when the protective haplotype of IL-23R (described in the above paragraph) was sequenced and compared to North American Caucasian CD patients, there was a one-nucleotide difference between Finnish (CCTGATCG) and North American (CGTGATCG) CD patients\cite{36}. In conclusion, the HLA-DRB1*0103 allele has been confirmed in CD patients, which shows an inherited susceptibility of colonic inflammation. The TNFRSF1A gene variants are markers of ileocolonic involvement in CD\cite{36}. Although this study shows a weaker association of the IL23R gene, it confirms the genetic involvement within a Finnish population.

In another population study among French-Canadian and English-Canadian children, the association between genetic variants of the IL23R gene and early-onset of CD has been investigated. To study the associations accurately, they have carried out both a case-control and a family-based study. They have targeted the 10 SNPs in IL23R achieved by the genome-wide study and the three CARD15 SNPs. In total, 259 CD patients and 139 controls were recruited with a mean age at diagnosis of 13.3 years (range: 2.6-20 years)\cite{36}. The IL23R protective allele, rs11209026, was only present in 2% of CD case chromosomes and 6% of the control chromosomes\cite{36}. All CARD15 variant allele frequencies were higher among CD patients when compared to the control group\cite{36}. In the IL23R gene, they observed a significant association among four SNPs that did not possess any CARD15 variants\cite{36}. Therefore, variants in the IL23R gene were associated with early-onset CD among Canadian children.
This study confirms previously reported findings in CD patients among North Americans.

In continuation of a study that was described in the ATG16L section of this paper, Latiano and colleagues have shown that the replication of IL23R is associated in adult and pediatric onset of IBD in Italy. Approximately 730 CD patients were genotyped for the rs7517847 and rs11209026 variants. For the rs7517847 polymorphism, significant reductions were found in minor allele (G) frequency in CD patients when compared with controls and in the number of carriers. No differences were found in UC patients. When the rs11209026 polymorphism was examined, a significant increase in the frequency of the risk genotype was observed in CD patients. In either SNP, there were no correlations between phenotypes of CD and risk alleles or genotypes of the IL23R gene.

To specify which IL23R variant is the main disease associated variant, a study using German CD patients was conducted. Among all 10 IL23R SNPs, they chose to focus their study on the rs1004819 variant because it had the strongest association to CD when compared to controls. This variant showed high prevalence of ileal involvement when carriers had the TT genotype versus the CC wild-type genotype. This identification is different from recent data published by Roberts et al., which identified the rs7517847 variant as having the strongest association with CD, along with other overlapping North American study populations. They have only analyzed ileal cases of CD, therefore, it is assumed that IL23R variants are predisposed to an ileal disease phenotype. In a recent British study, they could not identify any association between disease phenotype and IL23R variants. Interestingly, they found that the rs1004819 variant was 1000-fold weaker than that reported in the German study.

Not only are the IL23R variants highly associated with CD, they are also associated with other chronic inflammatory diseases, such as the rs10489629 variant with chronic periodontitis. In psoriasis, the variant rs11209026 has been described as a predisposing haplotype. In both CD and psoriasis, treatment with an anti-p40 IL-12/23 antibody has shown promising results. Antibodies to the p40 subunit block both IL-12 and IL-23, although in knockout mice studies, it has been proven that IL-23 drives chronic intestinal inflammation. Therefore, IL23R can serve as a therapeutic target in many different chronic inflammatory diseases, although the variants may differ among the different diseases. It is hypothesized that this is due to alternative mRNA splicing, which results in corresponding IL23R isoforms with different tissue distribution.

**IBD5 GENE**

The IBD5 gene is about 250 kb and is located at position 5q31. During a genome-wide linkage analysis, mapping studies have identified a risk haplotype within the IBD5 locus. Many studies have linked this gene and its two variants, SLC22A4 (OCTN1) and SLC22A5 (OCTN2), to CD. IBD is believed to originate from an uncontrolled mucosal immunity of the G1 tract. Although these variants are associated with CD, they act independently because there is no statistical evidence for interaction between IBD5 and the IL23R, ATG16L1 or CARD15 genes.

**CTLA4 VARIANTS**

The CTLA4 gene is a member of the immunoglobulin superfamily and is expressed on the surface of helper T cells. It is located within the 2q33 region, translates into a protein that plays a role in the immune system, and may have a genetic association with CD. It is a T-cell suppressor, which is essential in the function of the CD25 and CD4 regulatory cells. These regulator cells control the process of intestinal inflammation. Many SNPs have been studied within this gene and it has been found that the rs3087243 variant shows the strongest association with IBD followed by the rs11571302, rs7565213 and rs11571297 variants. Although studies have shown that the three variants in the CTLA4 gene, g.49A > G (rs231775), g.-318C > T (rs5742909), and rs3087243, have no association with CD, other work has suggested that these variants may control the phenotype of CD.

A study performed by Hradsky has shown no crude association between CD and SNPs within the CTLA4 gene. The study explored the possibility of interactions in CTLA4 SNPs with variants in IL23R and NOD2. The R-project package SNPassoc was used and significant interactions between the three CTLA4 variants with NOD2 p.Leu1007fsX1008 and IL23R rs11209026 were observed. This may be due to complex gene-gene interactions. To characterize further the different variants and whether they determine phenotype in CD patients, the study used a case-only design. They observed a difference in minor allele frequency at the rs3087243 gene between pediatric-onset and adult-onset of CD. It seems that a genetic factor has a greater impact in early-onset patients when compared to the adult-onset patients. Within this study, the age of diagnosis and localization of the disease was strongly associated with the rs3087243, rs11571302 and rs11571297 variants.

**NOD2, IL23R, OCTN1/2 AND ATG16L1 POLYMORPHISMS**

**Gene-gene interactions**

Interactions of the major IBD alleles show a high susceptibility in CD patients. Gene-gene interaction can either enhance or weaken the effects of an individual gene, therefore making it more important than independent studies looking into the effects of single susceptibility genes. Csongei and colleagues have performed a gene-gene interaction analysis in the Hungarian CD population. They concentrated on the two IL23R gene risk variants (rs2201841 and rs1004819), the ATG16L1 gene variant (Thr300Ala), and the three NOD2/CARD15 variants.
variants (rs2066844, L1007fs and rs2066845). Logistic regression analysis showed that the IL23R variants, both rs1004819 and rs2201841 and the NOD2/CARD15 variants (rs2066844 and L1007fs) conferred significant risk for CD. When the patients were homozygous for IL23R (rs1004819 and rs2201841) variants or ATG16L1, there was a highly increased risk for CD. When they analyzed possible statistical interactions between pairs of ATG16L1, IL23R and CARD15 variants, no evidence of interactions was found. Therefore, all examined loci contribute independently to CD risk. Although significant statistical interactions were not detected, these susceptibility factors may have a cumulative effect in the Hungarian population.

Further gene-gene interaction studies have been performed in another population study, as well as assessment of CD genetic risk factors. The study included five SNP variants for IL23R, the functional variants SLC22A4 and SLC22A5, and the ATG16L1 missense risk polymorphism Thr300Ala. All the SNPs, except for rs1495965 of IL23R, showed significant association when carriers were either homozygous or heterozygous for the alleles[5]. Among them all, rs10889677 of IL23R, showed the strongest association with CD risk[5]. Unsurprisingly, ATG16L1 also showed a strong association with CD[5]. None of the SNPs showed an association with UC. When they evaluated correlations between genotype and phenotype for intestinal complications, carriers of the rs7517848 allele of IL23R, particularly those who were homozygous for the allele, were found to be at risk for ileal disease. No other SNPs showed differences in genotype or carrier frequencies when compared to CD patients and their history of complications.

Homozygote variants from the IBD5 and ATG16L1 genes had a greater risk than heterozygotes, suggesting a gene dosage effect[5]. When SNPs were considered two at a time, the best interactions were shown between IL23R-rs10889677 and IBD5-rs17622208, which were not statistically significant[5]. However, when using a logistic regression approach between these two markers, the IBD5-rs17622208 risk was only significant in the presence of the IL23R-rs10889677 risk allele[5]. When SNPs were considered three at a time, the model suggested statistically significant interactions between IL23R-rs10889677, IBD5-rs11739135, and ATG16L1-rs2241880[5]. When SNPs were considered four at a time, the model suggested interaction between IL23R-rs2201841, IL23R-rs7517847, and IBD5-rs11739135[5]. A study in Oxford, UK has shown that certain IL23R polymorphisms have an association with CD only when the person is positive for IBD5[5]. In conclusion, the logistic regression model did not show any significant evidence of gene-gene interaction, due to the small size of the study, even though there was a consistently small association between IBD5 and IL23R.

**Childhood- vs adult-onset CD**

The pathogenesis of pediatric and adult IBD differ. The age of childhood and adult onset varies between cultures to culture because different cultures base it on different aspects such as physical, mental, or puberty. North American studies choose an age cut-off of 18 years, and < 18 years is considered a child, even though an individual at this age is physically mature. This is why Canadian studies have chosen to describe a child as < 16 years old. The belief is that the lower the age cut-off, the better the results are when comparing pediatric and adult onset of disease. Recent studies have shown that a subgroup of patients with early-onset IBD may have specific phenotypes that differ from adult-onset IBD[39]. Many believe that pediatric-onset IBD is influenced by genetics compared to adult onset because there is less time for exposure to environmental modifiers to influence the onset of disease. Adult-onset is probably due to a mixture of genetics and abundant environmental exposure[39]. For example, smoking is a major variable in adult IBD patients, but has little influence on pediatric IBD cohorts. Currently, there are conflicting studies on whether NOD2/CARD15 polymorphisms are associated with the age of onset of IBD because some show an association towards a younger age, while others show no effect[44].

A study by Gazoul et al[25] has investigated the main polymorphisms and their association with childhood-onset of CD in a Greek cohort. While investigating the genotype and allele frequencies of the NOD2/CARD15 polymorphisms, rs2066844, rs2066845 and 3020insC, a statistically significant association between rs2066844 and adult-onset CD was observed. In both pediatric- and adult-onset CD, individuals with at least one NOD2/CARD15 polymorphism showed a genotype-phenotype correlation with ileal involvement[25]. The study also confirmed the recently described association between IL23R variants in both child- and adult-onset CD[35]. There has been conflicting evidence in studies regarding the ATG16L1 SNPs. One study has shown no association with early-onset or adult-onset CD[35]. Recent research has indicated that the ATG16L1 rs2144880 variant is associated with adult-pediatric-onset CD, whereas other studies have demonstrated an association with diagnosis at an earlier age[35]. In conclusion, the 3020insC variant in the NOD2/CARD15 gene is associated with CD and occurs considerably more often in childhood- than in adult-onset patients with CD[35].

There is growing evidence that pediatric-onset IBD shows distinct differences when compared to its adult counterpart. Familial aggregation studies have shown an age-adjusted risk of developing IBD in first-degree relatives of affected individuals compared to the general population[35]. The risk increases to > 30% for children when both parents are affected with IBD, suggesting that family history is the strongest risk factor[35]. This is especially true among CD patients. Familial cases of CD occur at a younger age with greater severity than random sporadic cases[35]. One main difference observed between pediatric- and adult-onset is that early onset shows a distinct and
more aggressive phenotype, such as the need for surgery, than similar IBD in individuals > 20 years old.[40]

Although NOD2/CARD15 mutations are neither sufficient nor necessary for the development of IBD, they are associated with a younger age of onset, presence of ileal involvement, and the development of strictures.[49] There is also a gene dosage effect for CD location and complications. For example, stricture complications occur more frequently in CD children with the 1007fs mutation in the NOD2/CARD15 gene compared to children without this variant due to early surgery.[40]. Therefore, children with this mutation have a sixfold increased risk for developing a stricture complication[40].

There has been conflicting evidence among studies that have attempted to show a link between the IBD5 locus and early-onset CD. A study by Rioux has found that the IBD5 locus is associated with early-onset CD where children were defined with an age of onset of < 16 years old.[49]. However, studies from pediatric-onset CD cohorts have demonstrated that the risk of IBD5 is lower compared to that in adult-onset CD, while others have demonstrated enhanced risk of developing CD when an individual has both SLC22A4-A5 and NOD2/CARD15 mutations.[49]. This connection may be due to a common pathophysiological mechanism.[23].

Although there is no sex difference among patients with adult-onset IBD, there is a clear and distinct difference among children with CD. Many studies from pediatric CD cohorts in the United States, Canada and the United Kingdom have shown an increase in male incidence. The higher male to female ratio continues to be unexplained. These differences are not observed among UC pediatric patients.[23]. It seems that sex is an age-dependent variable that has more influence on children than adults with IBD.

There are also phenotypic differences, such as disease location, among children and adult CD. For example, increased rates of upper GI tract disease and pure colonic disease in pediatric-onset CD have been identified. This difference may be due to the amount of examination during the onset of disease. Children undergo extensive GI endoscopy, whereas adults do not.[23]. These findings may be artificial or represent a true disease distinction among children and adults. Another distinction is the occurrence of colon-predominant disease during childhood-onset IBD under the age of 10 years old. With children < 5 years old, all have colon-only disease.[41].

During a study of approximately 1400 North American early-onset patients, data showed a colon-predominant phenotype in children < 8 years old. In another study in Europe, the acquisition of ileal CD became increasingly common as an individual approached 16 years old.[23]. These data confirm an association of colon-predominant phenotype in early diagnosed children that changes as they grow older.

**Population studies with NOD2, IL23R and ATG16LI polymorphisms**

NOD2, IL23R and ATG16LI polymorphisms were studied in a Lithuanian cohort with IBD. The study included 57 unrelated patients with CD, 123 with UC and 186 healthy individuals as controls. The three NOD2 variants, the IL23R variant rs11209026, and the ATG16L1 variant Thr300Ala, were genotyped among the population sample. No individuals were carriers of all three NOD2 risk alleles, whereas two CD patients were compound heterozygotes[19]. Carriers of at least one NOD2 variant were highest among CD patients[19]. There were no significant differences observed between UC patients and the controls. The NOD2 variant, Leu1007insC, was significantly associated with increased susceptibility in the Lithuanian CD population[19]. In comparison to the other two NOD2 variants, the frequencies were very low and not significant in controls and among the IBD patients[18]. In contrast to other European studies, a positive association between rs2066844, rs2066845 and CD was not found[19]. When they further tried to analyze II.23R and ATG16L1, they were unable to replicate previous findings of increased susceptibility to IBD within the Lithuanian population[19]. They were a trend for a possible association with the ATG16L1 risk allele[19]. It is of particular interest to study this population because Baltic countries have low IBD incidence rates, especially for CD. To confirm distinct IBD subtypes, a study using a larger North-Eastern European IBD sample needs to be investigated.

Another population study was performed among New Zealand Caucasians with IBD. Their cohort included 466 UC patients, 496 CD patients and 591 controls. All individuals were genotyped for the II.23R rs11209026 SNP, the ATG16L1 rs2241880 SNP, and the CARD15 variants. Significant interaction was detected between variants in ATG16L1, II.23R and CARD15 and CD susceptibility, whereas no significant association was observed between II.23R or ATG16L1 genotypes and IBD sub-phenotypes[37]. The strongest association occurred between the ATG16L1 rs2241880 variant and CD, with no association detected with UC[37]. ATG16L1 is suggested to have a CD-specific susceptibility locus. Unlike ATG16L1, the II.23R rs11209026 variant was strongly associated with both CD and UC. In their patient cohort, there was no evidence that II.23R or ATG16L1 genotypes influenced disease behavior, age of onset, location, or the need for surgical bowel resection[37]. On the other hand, CARD15 was consistently a susceptibility factor and predictor of CD phenotype. Similar to many other studies, all three CARD15 SNPs are significantly overrepresented in patients with IBD family history, early onset of disease, ileal disease involvement, and development of complications[33].

**Interactions between NOD2 and IL23R variants with toll-like receptor-9 polymorphisms**

Toll-like receptors (TLRs) are single, membrane-spanning proteins that play a key role in the innate immune system. These receptors recognize microbes that have structurally conserved molecules that breach the physical
The responsiveness of the GI tract to luminal bacteria is dependent on the interaction of transmembrane TLRs and the intracellular NOD2 receptor\[^{19}\]. Specifically, TLR9 plays a role in the maintenance of intestinal homeostasis and inflammation in IBD. TLR9 is also responsible for stimulating NOD2, which in turn enhances innate immune responses. Patients with two NOD2 mutations lose the synergistic affect between NOD2 and TLR9 stimulation. Therefore, interactions of both receptors have implications for intestinal homeostasis and inflammation\[^{19}\]. The TLR9 gene is located on chromosome 3p21.3, which is close to other CD susceptible loci\[^{19}\]. There are four SNPs in TLR9, but two of them are sufficient to distinguish between the haplotypes, which are rs5743836 and rs352140.

There might be a synergistic effect of NOD2 and TLR9 stimulation, therefore, Torok and colleagues have tested for gene interactions between TLR9 and CD-associated variants of NOD2\[^{18}\]. Significant associations between the two were observed that were specific to CD\[^{18}\]. The controls and UC showed no difference in distribution of TLR9 polymorphisms and NOD2 variants\[^{18}\]. Other CD variants in IL23R, ATG16L1 and IBD5 were analyzed for epistatic interactions. Aside from NOD2, the most significant association was found in the IL23R variant rs1004819, with ATG16L1 showing weaker associations with CD. There was no significant association between TLR9 polymorphisms with CD or UC phenotypes\[^{18}\]. Along with previous studies, they also showed that NOD2 mutations were associated with younger age of diagnosis of CD, ileal disease, and need for surgery\[^{18}\]. When there were two NOD2 mutations, there was a higher frequency of penetrating disease\[^{18}\].

In conclusion, a new association between CD and a TLR9 polymorphism has been found. There is evidence that when CD patients carry CD-associated NOD2 variants, they have an increased incidence of TLR9 polymorphisms. This is not surprising because there is a synergistic effect of NOD2 and TLR9 stimulation and it is important for the maintenance of intestinal homeostasis and inflammation. TLR9 also demonstrates significant epistatic interactions with IL23R variants, but unlike NOD2, there is no association with the frequency in TLR9 polymorphisms present. This study shows the first evidence for interaction between polymorphisms in TLR9 and variants between NOD2 and IL23R\[^{18}\].

### Table 1 Key gene polymorphisms and their significance in Crohn’s disease

| Gene      | Polymorphism                    | Relationship significance                                          | Ref. |
|-----------|---------------------------------|-------------------------------------------------------------------|------|
| ATG16L    | rs2241880                       | Associated with ileal form of CD with or without colonic involvement | [4,12] |
| NOD2/CARD15 | rs2066844, rs2066845, 3020insC, 1007fs | Independently associated with CD                                 | [20] |
| IB5       | SLC22A4 (OCTN1), SLC22A5 (OCTN2) | Independently associated with CD                                 | [12] |
| CTLA4     | rs3087243, rs11571302, rs11571297, rs2565213 | Associated with IBD; no crude association to CD                  | [33] |
| TNFSF15   | 80,000 SNPs tested, including 7 SNPs within a 280 kb region on chromosome 9q32 | Strongly associated with CD for Japanese and Jewish cohorts, but not for Europeans | [4,15,16] |
| IL23R     | rs11209026, rs1004819           | Highly associated with CD                                         | [4,33] |

SNPs: Single-nucleotide polymorphism; CD: Crohn’s disease.

**CONCLUSION**

CD is an autoimmune disease characteristic of chronic intestinal inflammation and lesions. It can affect people of all ages and ethnicities worldwide. Recent genome-wide studies have shown significant genetic associations of several variants and susceptibility to CD. This is true for North American, South American and European populations. Certain variants have been linked only to Asian and African cohorts. It is vital to understand the pathology of CD and the underlying genetic interactions to increase efficiency of diagnosis and develop drugs that target specific immune system pathways. There have been several genetic variants highly associated with CD, which include ATG16L1, TNFSF15, NOD2/CARD15, IL23R and IBD5 (Table 1).

The process of autophagy is an important aspect of our immune system. It is a way to destroy foreign pathogens that enter the body. The Thr300Ala variant within the N terminus of ATG16L, which is part of a complex that forms autophagosomes, has significant associations with CD. Different ethnicities show different genotype markers. This is seen in TNFSF15, which is involved in systemic inflammation and regulation of various immune cells. Seven haplotypes have been identified within a Japanese cohort, but not in other ethnic cohorts. More research needs to be conducted to characterize fully this possible genetic link to CD. The most significant association with CD is observed among NOD2/CARD15 and IL23R variants. NOD/CARD15 plays an essential role in maintaining the intestinal normal flora. There are many theories about the cause of CD, and one of them includes a shift in the intestinal bacterial flora. Therefore, it is no surprise that NOD2 variants are highly associated with CD. There are three independent variants associated with CD: rs2066844, rs2066845 and 1007fs. The other highly associated genetic link is observed within the IL23R gene. One variant that is highly associated with CD is rs1004819, whereas rs11209026 confers protection against CD. IL23R plays an essential role in barriers. Once bound, TLRs activate the immune system.

References:

[18] Naser SA et al. Genetics in Crohn’s disease. WJG 2012;18(2):340-353

[19] Shcherbina A, Cullender T, Lissens W, et al. NOD2/CARD15 plays an essential role in maintaining the intestinal normal flora. TLR9 is also responsible for stimulating NOD2, which in turn enhances innate immune responses. Patients with two NOD2 mutations lose the synergistic affect between NOD2 and TLR9 stimulation. Therefore, interactions of both receptors have implications for intestinal homeostasis and inflammation. The TLR9 gene is located on chromosome 3p21.3, which is close to other CD susceptible loci. There are four SNPs in TLR9, but two of them are sufficient to distinguish between the haplotypes, which are rs5743836 and rs352140.

There might be a synergistic effect of NOD2 and TLR9 stimulation, therefore, Torok and colleagues have tested for gene interactions between TLR9 and CD-associated variants of NOD2. Significant associations between the two were observed that were specific to CD. The controls and UC showed no difference in distribution of TLR9 polymorphisms and NOD2 variants. Other CD variants in IL23R, ATG16L1 and IBD5 were analyzed for epistatic interactions. Aside from NOD2, the most significant association was found in the IL23R variant rs1004819, with ATG16L1 showing weaker associations with CD. There was no significant association between TLR9 polymorphisms with CD or UC phenotypes. Along with previous studies, they also showed that NOD2 mutations were associated with younger age of diagnosis of CD, ileal disease, and need for surgery. When there were two NOD2 mutations, there was a higher frequency of penetrating disease.

In conclusion, a new association between CD and a TLR9 polymorphism has been found. There is evidence that when CD patients carry CD-associated NOD2 variants, they have an increased incidence of TLR9 polymorphisms. This is not surprising because there is a synergistic effect of NOD2 and TLR9 stimulation and it is important for the maintenance of intestinal homeostasis and inflammation. TLR9 also demonstrates significant epistatic interactions with IL23R variants, but unlike NOD2, there is no association with the frequency in TLR9 polymorphisms present. This study shows the first evidence for interaction between polymorphisms in TLR9 and variants between NOD2 and IL23R.

**CONCLUSION**

CD is an autoimmune disease characteristic of chronic intestinal inflammation and lesions. It can affect people of all ages and ethnicities worldwide. Recent genome-wide studies have shown significant genetic associations of several variants and susceptibility to CD. This is true for North American, South American and European populations. Certain variants have been linked only to Asian and African cohorts. It is vital to understand the pathology of CD and the underlying genetic interactions to increase efficiency of diagnosis and develop drugs that target specific immune system pathways. There have been several genetic variants highly associated with CD, which include ATG16L1, TNFSF15, NOD2/CARD15, IL23R and IBD5 (Table 1).

The process of autophagy is an important aspect of our immune system. It is a way to destroy foreign pathogens that enter the body. The Thr300Ala variant within the N terminus of ATG16L, which is part of a complex that forms autophagosomes, has significant associations with CD. Different ethnicities show different genotype markers. This is seen in TNFSF15, which is involved in systemic inflammation and regulation of various immune cells. Seven haplotypes have been identified within a Japanese cohort, but not in other ethnic cohorts. More research needs to be conducted to characterize fully this possible genetic link to CD. The most significant association with CD is observed among NOD2/CARD15 and IL23R variants. NOD/CARD15 plays an essential role in maintaining the intestinal normal flora. There are many theories about the cause of CD, and one of them includes a shift in the intestinal bacterial flora. Therefore, it is no surprise that NOD2 variants are highly associated with CD. There are three independent variants associated with CD: rs2066844, rs2066845 and 1007fs. The other highly associated genetic link is observed within the IL23R gene. One variant that is highly associated with CD is rs1004819, whereas rs11209026 confers protection against CD. IL23R plays an essential role in
mediating proinflammatory activities. Another highly associated genetic link with CD is observed in two variants within IBD5. The two variants, SLCL22A4 (OCTN1) and SLCL22A5 (OCTN2), show significant association with CD, but no interactions between these variants and other CD-associated genetic variants have been observed. The last highly associated genetic link with CD is observed within the CTLA4 gene. This gene plays a role in the immune system. The four polymorphisms associated with IBD are rs3087243, rs11571302, rs7565213 and rs11571297.

Many CD variants are associated with a specific CD phenotype, age of diagnosis, severity and location of CD, and surgical outcome. Gene-gene interactions have also been characterized among the different variants and other immune system receptors. We need to understand fully the pathogenesis of CD to target pathways for effective treatment. Further research is needed to explore all possible gene-gene interactions due to a gene dose effect associated with CD. It seems that new genetic associations are constantly being uncovered within IBD and then associated with either CD or UC. Overall, CD shows a greater genetic link than UC. Recent discoveries have led to therapies and treatments that show much promise. Researchers need to continue the search for genetic links and diseases because this may be the only way to understand the pathology of CD and develop effective treatments.

REFERENCES

1. Brand S, Staudinger T, Schnitzler F, Pfennig S, Hofbauer K, Dambacher J, Seiderer J, Tillack C, Konrad A, Crispin A, Göke B, Lohse P, Ochskenkühn T. The role of Toll-like receptor 4 Assp299Gly and Thr399Ile polymorphisms and CARD15/NOD2 mutations in the susceptibility and phenotype of Crohn’s disease. Inflamm Bowel Dis 2005; 11: 645-652
2. Cummings JR, Ahmad T, Geremia A, Beckly J, Cooney R, Hancock L, Pathan S, Guo C, Cardon LR, Jewell DP. Contribution of the novel inflammatory bowel disease gene IL23R to disease susceptibility and phenotype. Inflamm Bowel Dis 2007; 13: 1063-1068
3. Cho JH. Inflammatory bowel disease: genetic and epidemiologic considerations. World J Gastroenterol 2008; 14: 338-347
4. Libioulle C, Louis E, Hansoul S, Sandor C, Farnir F, Franchimont D, Vermeulen S, Dewit O, de Vos M, Dixon A, Demarche B, Gut I, Heath S, Foglio M, Liang L, Laukens D, Mni M, Zelenika D, Van Gossum A, Rutgeerts P, Belaiche J, Lathrop M, Georges M. Novel Crohn disease locus identified by genome-wide association mapping in the HapMap CEU. PLoS Genet 2007; 3: e58
5. Csögei V, Járomi L, Sáfrány E, Sipéky C, Magyari L, Farrago B, Bogdányi I, Polgár N, Lakner L, Sarlós P, Varga M, Melegh B. Interaction of the major inflammatory bowel disease susceptibility alleles in Crohn’s disease patients. World J Gastroenterol 2010; 16: 176-183
6. Beattie RM, Cotro NM, Fell JM, Afzal NA, Heuschkel RB. Inflammatory bowel disease. Arch Dis Child 2006; 91: 426-432
7. Repasky EA, Malmström V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. J Exp Med 2000; 192: 295-302
8. Fujita N, Itoh T, Omori H, Fukuda M, Noda T, Yoshimori T. The Atg16L1 complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. Mol Biol Cell 2008; 19: 2092-2100
9. Tremelling M, Cummings F, Fisher SA, Mansfield J, Gwilliam R, Keniry A, Nimmor ER, Drummond H, Onnie CM, Prescott NJ, Sanderson J, Bredin F, Berzunzi C, Forbes A, Lewis CM, Cardon L, Dalakas P, Jewell D, Mathew CG, Parkes M, Satsangi J. IL23R variation determines susceptibility but not disease phenotype in inflammatory bowel disease. Gastroenterology 2007; 132: 1657-1664
10. Sventoraityte J, Zvirbliene A, Franken A, Kwaistkowski R, Kiudelis G, Kupcinis L, Schreiber S. NOD2, IL23R and ATG16LI polymorphisms in Lithuanian patients with inflammatory bowel disease. World J Gastroenterol 2010; 16: 359-364
11. Rioux JD, Xavier RJ, Taylor KD, Silverberg MS, Gyotte P, Huettt 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, Steinhardt AH, Rotter JI, Duerr RH, Cho JH, Daly MJ, Brant SR. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat Genet 2007; 39: 596-604
12. Hadrsky O, Dusatkova P, Leneic M, Bronsky J, Neovaral J, Vitek L, Lukas Z, Zeniskova I, Cinek O. The CTLA4 variants may interact with the IL23R and NOD2-confessed risk in development of Crohn’s disease. BMC Med Genet 2010; 11: 91
13. Prescott NJ, Fisher SA, Franke A, Hampe J, Onnie CM, Soars D, Bagnall R, Mirza MM, Sanderson J, Forbes A, Mansfield JC, Lewis CM, Schreiber S, Mathew CG. A non-synonymous SNP in ATG16L1 predisposes to ileal Crohn’s disease and is independent of CARD15 and IBD5. Gastroenterology 2007; 132: 1665-1671
14. Xia B, Crusius JB, Wu J, Zwiers A, van Bodegraven AA, Perä AS. CTLA4 gene polymorphisms in Dutch and Chinese patients with inflammatory bowel disease. Scand J Gastroenterol 2002; 37: 1296-1300
15. Yamazaki K, McGovern D, Ragoussis J, Paolucci M, Butler H, Jewell D, Cardon L, Takaoze M, Tanaka T, Ichimori T, Saito S, Sekine A, Iida A, Takahashi A, Tsunoda T, Lathrop M, Nakamura Y. Single nucleotide polymorphisms in TNFSF15 confer susceptibility to Crohn’s disease. Hum Mol Genet 2005; 14: 3499-3506
16. Michelsen KS, Thomas LS, Taylor KD, Yu QT, Mei L, Landers CJ, Derkowski C, McGovern DP, Rotter JL, Targan SR. IBD-associated TL1A gene (TNFSF15) haplotypes determine increased expression of TL1A protein. PLoS One 2009; 4: e4719
17. Van Limbergen J, Russell RK, Nimmor ER, Ho GT, Arnott ID, Wilson DC, Satsangi J. Genetics of the innate immune response in inflammatory bowel disease. Inflamm Bowel Dis 2007; 13: 338-355
18. Torör HP, Gläs J, Endres I, Tonenchl L, Teshome MY, Wetzeke M, Klein W, Lohse P, Ochskenkühn T, Folwaczny M, Göke B, Folwaczny C, Mükler-Mýhosk B, Brand S. Epistasis between Toll-like receptor-9 polymorphisms and variants in NOD2 and IL23R modulates susceptibility to Crohn’s disease. Am J Gastroenterol 2009; 104: 1723-1733
19. Kobayashi KS, Cha'amallard M, Ogura Y, Henegariu O, Inohara N, Nuñez G, Flavell RA. Nod2-dependent regulation of the IBD-associated TL1A gene (TNFSF15) haplotypes determines increased expression of TL1A protein. PLoS Genet 2009; 5: e1000598
20. Vermeire S, Wild G, Kocher K, Cousineau J, Dufresne L, Bilton A, Langelier D, Pare P, Lapointe G, Cohen A, Daly MJ, Rioux JD. CARD15 genetic variation in a Quebec population: prevalence, genotype-phenotype relationship, and haplotype structure. Am J Hum Genet 2002; 71: 74-83

Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stocke
Naser SA et al. Genetics in Crohn’s disease

Serum levels are increased in Crohn’s disease and correlate with disease activity and IL23R genotype status. Inflamm Bowel Dis 2008; 14: 204-212

Oliver J, Rueda B, López-Nevo MA, Gómez-García M, Martin J. Replication of an association between IL23R gene polymorphism with inflammatory bowel disease. Clin Gastroenterol Hepatol 2007; 5: 977-981, 981.e1-2

Van Limbergen J, Russia RK, Nimmo ER, Drummmond HE, Smith L, Anderson NH, Davies G, Gillett PM, McGrogan P, Weaver LT, Bisset WM, Mahdi G, Arnott ID, Wilson DC, Satsangi J. Autophagy gene ATG16L1 influences susceptibility and disease location but not childhood-onset in Crohn’s disease in Northern Europe. Inflamm Bowel Dis 2008; 14: 338-346

Lappendainen M, Halme L, Turunen U, Saavalainen P, Einansdotter E, Färkkilä M, Mussack T, Cardon LR, Jewell DP. Confirmation of the role of ATG16L1 as a Crohn’s disease susceptibility gene. Inflamm Bowel Dis 2008; 14: 1118-1124

Roberts RL, Geary RB, Hollis-Moffatt JE, Miller AL, Reid J, Abkevich V, Timms KM, Gutin A, Lanchbury JS, Merriman TR, Barclay ML, Kennedy MA. 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 2007; 102: 2754-2761

Cummings JR, Cooney R, Pathan S, Anderson CA, Barrett JC, Beckly J, Geremia A, Hancock L, Guo C, Ahmad T, Cardon LR, Jewell DP. Confirmation of the role of ATG16L1 as a Crohn’s disease susceptibility gene. Inflamm Bowel Dis 2007; 13: 943-946

Mamula P, Telega GW, Markovitz JE, Brown KA, Russo PA, Piccoli DA, Baldassano RN. Inflammatory bowel disease in children 5 years of age and younger. Am J Gastroenterol 2002; 97: 2005-2010

Russell RK, Drummmond HE, Nimmo EE, Anderson N, Smith L, Wilson DC, Gillett PM, McGrogan P, Hassan K, Weaver LT, Bisset M, Mahdi G, Satsangi J. Genotype-phenotype analysis in childhood-onset Crohn’s disease: association of IL23R, TNFRSF1A, and HLA-DBR1*0103 allele variants with inflammatory bowel disease phenotypes in the Finnish population. Inflamm Bowel Dis 2008; 14: 1125-1130

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

Newman B, Gu X, Wintle R, Cescos D, Yazdanpanah M, Liu X, Peletkova V, Van Oene M, Amos CI, Siminovich KA. A risk haplotype in the Solute Carrier Family 22A4/22A5 gene cluster influences phenotypic expression of Crohn’s disease. Gastroenterology 2005; 128: 260-269

Heyman MB, Kirschner BS, Gold BD, Ferry G, Baldassano R, Cohen SA, Winter HS, Fain P, King C, Smith T, El-Serag HB. Children with early-onset inflammatory bowel disease (IBD): analysis of a pediatric IBD consortium registry. J Pediatr 2005; 146: 35-40

S-Editor Tian L · L-Editor Kerr C · E-Editor Li JY