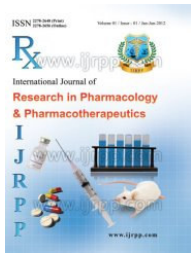
International Journal of Research in Pharmacology & Pharmacotherapeutics



ISSN Print: 2278-2648 IJRPP |Vol.10 | Issue 1 | Jan - Mar - 2021
ISSN Online: 2278-2656 Journal Home page: www.ijrpp.com

Research article Open Access

Role of the card 15 gene, R702w mutation in inflammatory bowel disease individuals

Dr.A.Jyothy[1] , Dr.M.Sujatha[2], Mrs.Deepika[3]

[1]*Director,Institute Of Genetics and Hospital for Genetic Diseases,Osmania University, Hyderabad, India.*

[2]*M.B.B.S.,D.C.H,PhD.,Head,Dept.of.Clinical Genetics.*
[3]*(JRF-Clinical Genetics)*

***Corresponding author: K.Praveena**

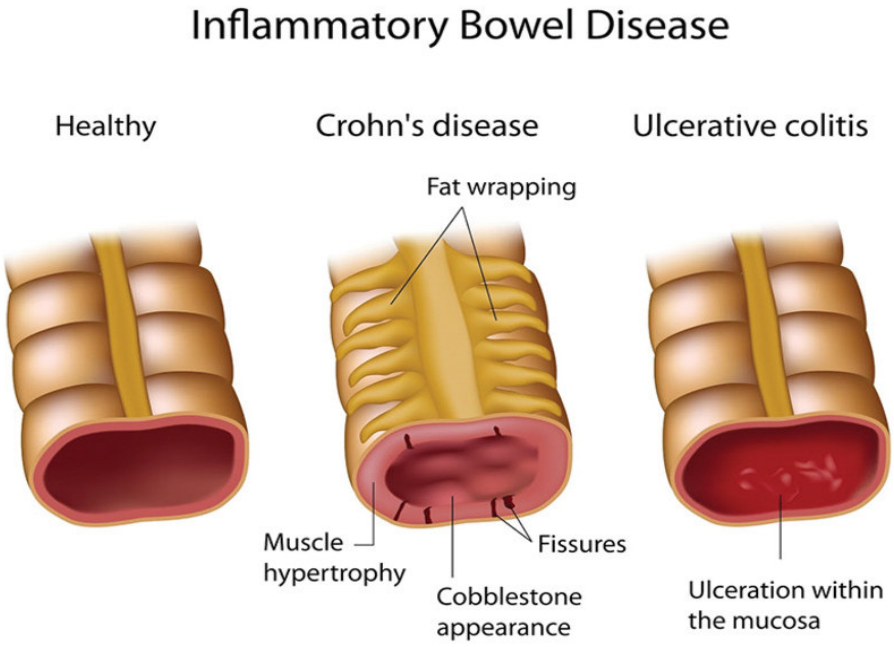
ABSTRACT

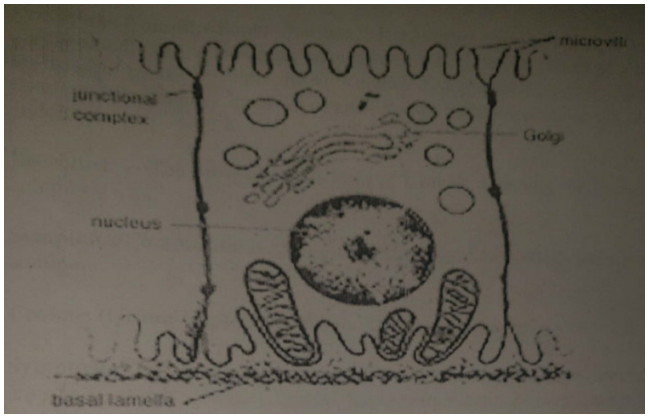
Digestive problems are among the most common conditions affecting population today. There are many different types of digestive problems, from gastrointestinal infections that make a person miserable surpass quickly to long term illness like IBD. IBD is a general term that refers to illness that causes chronic inflammation in the intestines. IBD consists mainly of 2 conditions. (1) Ulcerative colitis and (2) Crohn`s disease.

CARD15 wild type protein mediates responsiveness to lipopolysaccharide (LPS) and peptidoglycan (PGN) preparations from several bacteria. Recent works identified muramly dipeptide (MDP), the moiety specifically recognized by CARD15. The 3 major crohn disease associated variants are defective in their ability to activate NF-KB in response to MDP.

The main objective of this work is to screen the R702W mutation in IBD individuals by using methods like DNA isolation, Amplification Refractory Mutation System (ARMS) (allele specific method) and electrophoresis. In this present study, the results revealed that there is no R702W variant polymorphism in all selected 20 IBD individuals.

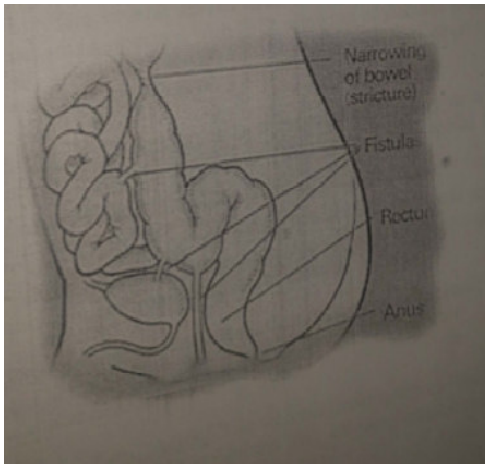
INTRODUCTION





Epithelial cells line the intestine and are one of several cell types in the lining of the intestine to be affected in ulcerative colitis or Crohn's disease. Among monozygotic twins, concordance ranges between 42% and 58% (100% would indicate that only genetic factors determined disease expression). Among dizygotic twins, concordance ranges between 0% and 12% a range of the same order of magnitude as that among non-twin siblings.

Crohn's disease susceptibility genes have been located at IBD on chromosomes 16 and 6p. The first gene specifically identified with Crohn's is the mutated NOD2 gene, which is localized to a linkage region on chromosome 16. It occurs 2 to 3 times as frequently in persons with Crohn's disease as in the general population. It appears to lead to an exaggerated immune response and is associated with the development of fibrostenotic disease.



Jewish populations, rates are higher in Ashkenazi Jews (of European descent). These differences occur across different time periods and geographic suggesting a genetic basis as the most likely explanation for these findings.

Background

Crohn`s disease (CD) and Ulcerative colitis(UC) are idiopathic, inflammatory disorders of the gastrointestinal tract.

Incidence

Prevalent in early childhood 100-200 per 100000 individuals. Children of South Asian population have high incidence.

IBD

IBD is most common chronic gastro- intestinal illness in children and adolescents. It is characterized by chronic intestinal inflammation with clinical symptoms such as diarrhea,bleeding,abdominal pain,fever,joint pain and weight loss. It has 2 forms Crohn`s disease and Ulcerative colitis.Ulcerative colitis involves inflammation of the colon and rectum.Crohn`s disease impacts a greater area of the upper intestinal digestive tract likely to trigger mal-absorption with chronic vitamin and nutrient deficiencies. IBD involves both small and large bowel.

Age of onset

Onset is between 10-30 years. Males and females are affected equally.

Crohn`s disease

Crohn`s disease is a disorder of the gastro intestinal tract. Affects any portion from lips to anus.Involves distal small bowel and colon. It produces a small ulcer over a lymphoid follicle to a deep fissure, ulcer ,scarring and inflammation.

Types

1. Gastroduodenal CD: Affects stomach and duodenum. Symptoms are weight loss,loss of appetite,nausea and vomiting. Identified as ulcer disease.
2. Jejunoileitis: Patchy areas of inflammation in the jejunum. Symptoms are abdominal pain and cramps. Fistulas ,diarrhea malabsorption which leads to weight loss and mal nutrition .
3. Ileitis: Affects the ileum. Symptoms diarrhea,cramping, Iron and vitamin-B12 deficiency, fistulas.
4. Ileocolitis: The most common form of Crohn`s disease affecting ileum and colon. Symptom is weight loss.
5. Crohn`s (Granulomatous) Colitis: Affects the colon only. Symptoms are diarrhea and rectal bleeding.

Pathophysiology

Crohn`s disease is an immune system dysfunction caused by an imbalance between production of pro-inflammatory cytokines and anti-inflammatory cytokines. Excessive activation of mucosal Tcells leads to Tran's luminal inflammation which is amplified and perpetuated by the release of pro-inflammatory cytokines and soluble mediators.

Pathogenesis

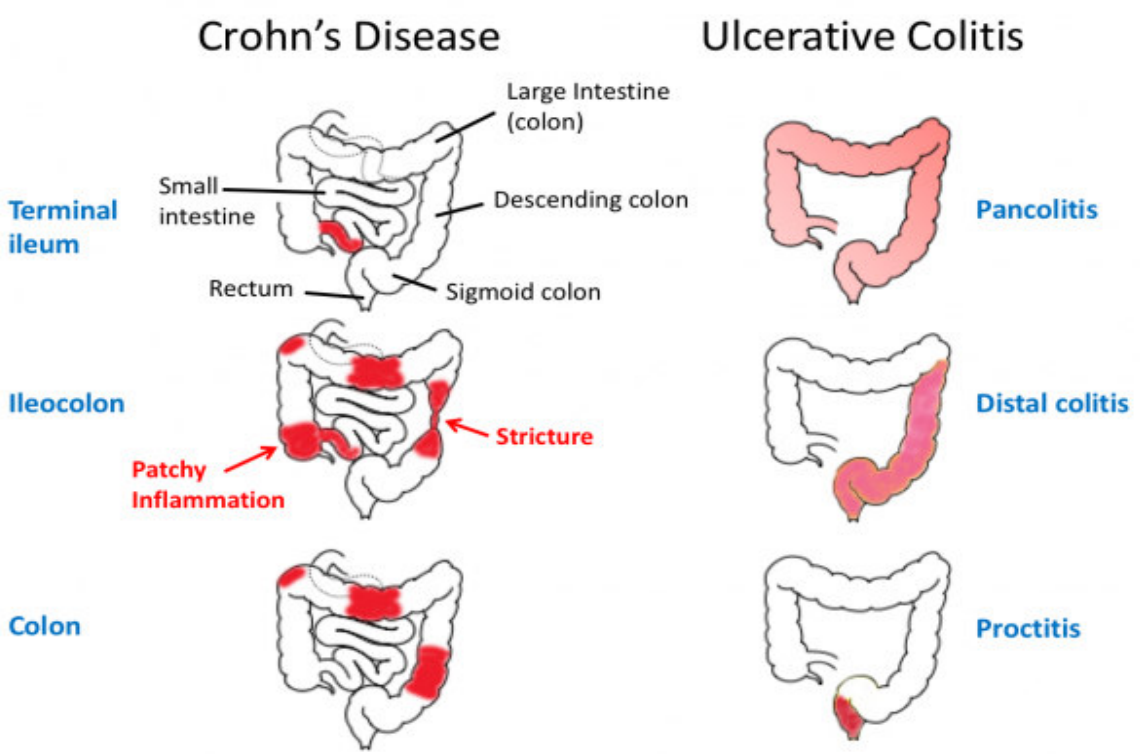
Both genetic susceptibility and environmental triggers contribute to the onset of Crohn`s disease. Environmental triggers include smoking,stress,antibiotics,non-steroidal inflammatory drugs,inter current infection and dietary factors. Crohn`s disease susceptible genes located at IBD1 on chromosome16 and6p. First gene identified with CD is mutated NOD2 / CARD15 gene. This gene acts as an intracellular receptor for the bacterial components including lipopolysaccharide.NOD2 gene is expressed in peripheral monocytes and structurally related to that plant R proteins.3major coding regions polymorphisms in NOD2gene. 3major CD variants exhibit a deficit in NF-kappa B activation in response to bacterial components.IBD2 on chromosome 12q and IBD3 on chromosome5q.

Complications of IBD

Arthritis,eye inflammation,liver diseases,skin disorders,mal -absorption , mal- nutrition, toxic mega colon,Bleeding due to ulcers in colon,intestinal cancers ,joints(stiffness),bones (calcium loss and low body weight),anemia,gallbladder disorders,blood clots,lung involvement ,mouth sores,delayed growth&development in children,neurologic complications,measles and autism.

Treatment

IBD children 5-ASA (AMINOSALICYLATES) and sulfasalazine orally or rectally Steroids such as prednisone and prednisolone.Azolothioprine, 6mercaptopurine and cyclosporine.Surgery or removal of colon.



Ulcerative colitis (UC)

Inflammatory disease of large intestine (colon)

Onset

People of 14-40 years

Symptoms

Inflammation and ulceration of innermost lining of the colon, bloody diarrhea, abdominal pain, knee pain, Ankle&joints pain,affect lower part of the colon-rectum(proctitis)Fever,nausea,vomiting and weight loss.

Classification of UC

1. Proctitis
2. Proctosigmoiditis
3. Left side colitis
4. Pancolitis

Factors associated for UC

Smoking and depression

Diagnosis

Physical examination,blood tests and samples of a bowel

Complications

Inflammation of eye,liver,skin rashes,anemia,kidney stones and arthritis

Treatment

Anti -inflammatory drugs,immune suppressive agents and surgery.

Genes linked to IBD

The first gene associated with Crohn's disease, the NOD2 gene was identified. On-going research is looking into how defects in the NOD2 gene leads to Crohn's disease and finding the other genes that cause IBD (it is likely that there are 4-5 genes involved). This could in turn be of potential benefit in predicting the course of disease in individual patients and in guiding appropriate medical therapy.

The inflammatory response

The immune system's infection fighters the primary infection-fighting units are two types of white blood cells. Lymphocytes and leukocytes. Lymphocytes include two sub-types known as T-cells and B-cells. Both types of cells are designed to recognize foreign invaders (antigens) and to launch an offensive or defensive action against them.

1. B-cells produce antibodies, which are separate agents that can either ride along with a B-cell or travel on their own to attack the antigen.
2. T-cells have special receptors attached to their surface that recognize the specific antigen.

T-cells are further categorized as killer T-cells or helper T-cells(TH cells)

1. Killer T-cells directly attack antigens that occur in any cells that contain a nucleus.
2. Helper T-cells also recognize antigens.

They stimulate B-cells and other white cells to attack the antigen. They also produce cytokines, powerful immune factors that have an important role in the inflammatory process.The actions of the helper T-cells are of special interest in inflammatory bowel disease.

- TH-cells stimulate other white blood cells called B-cells to produce antibodies. In this case, however, they appear to direct the B-cells to produce auto antibodies, which are directed against the body's own cells
- TH-cells also secret or stimulate the production o powerful immune factors called cytokines. In small amounts, cytokines are indispensable for healing. If overproduced, however, they can cause serious damage, including inflammation and cellular injury Cytokines, particularly specific ones known as tumor necrosis factor, interferon-gamma, and interleukins, cause intestinal inflammation and damage, which in a vicious cycle, attract even more helper-T cells.

Helper T-cells are further categorized as TH1 and TH2. An imbalance in these two types appears to occur in IBD although each disorder has a different balance.

- Ulcerative colitis patients favor a Th2 response, which activates the interleukins IL-5, and IL-10, which mostly affect mucosal areas in the intestine.
- Research indicates that Crohn's disease patients have increased activity in Th1 helper cells, which activates interleukin-2 (IL-2) and interferon-gamma, which affect intestinal cells. Tumor necrosis factor may be a particularly potent immune factor in Crohn's disease. It is important in properties that regulate inflammation and cell proliferation. If genetic or other factors increase production of this immune compound. It can lead to great harm.

Interleukin 6 appears to play a part in both IBDs, by inhibiting a natural mechanism called apoptosis, a process whereby cells self-destruct in such cases, cells proliferate faster than they die, causing an excessively strong immune response.

Adhesion Molecules increased levels of certain molecules called E-selection and intercellular adhesion molecule-1(ICAM-1) also appear to play a major role in the inflammatory process by causing damaging immune factors to accumulate on intestinal

cells. E-selection may be involved in the early stages of the disease (especially ulcerative colitis) and ICAM-1 in the persistence of either inflammatory bowel disease.

Matrix metalloproteinase greater activity of enzymes has been detected in the colons of patients with IBD such increased levels tend to break down the extracellular matrix, a barrier.

Causes

The causes of the inflammatory bowel diseases are unknown. Research has identified three contributing factors. The bacteria that reside normally in the bowel, a defect in the 'barrier' mechanism of the mucosal lining (possibly due to a genetic susceptibility) and poorly regulated or overly aggressive immune response of the mucosal lining. The MGH IBD center has taken a leading role in research for the identification of the causes of disease as well as disease management.

Other causes for inflammatory bowel disease

Infectiouscauses for IBD generally have a more acute onset and run a shorter course than idiopathic forms of IBD. Bacterial organisms that can produce IBD include Shigella, Salmonella, Campylobacter, and some E.coli. Bacteria are a common cause of acute self-limited colitis active IBD without chronic changes.

Viral etiologies include Norwalk-like virus and rotavirus (small bowel) as well as cytomegalovirus (CMV) and herpes simplex virus in immune compromised persons.

Other causes include chlamydial infection and amebiasis.Antibiotic associated IBD can occur from therapy with broad spectrum antibiotics leading to overgrowth of clostridium difficile or other organisms such as candida.This produces a toxin which causes mucosal damage (pseudomembranous colitis).

An IBD can also occur with ischemia. A less common disease is collagenous colitis, which is seen as a chronic watery diarrhea in middle-aged women and is characterized by lymphocytic inflammation of surface epithelium and thickened sub-epithelial collagen.

Genes Associated

About 20% of cases of Crohn's disease appear to run in families. It is a 'complex trait' which means that several genes at different locations in the genome may contribute to the disease.

A susceptibility locus for the disease was recently mapped to chromosome 16 candidate genes found in this region.

It includes several genes involved in the inflammatory response, including CD19 involved in B-lymphocyte function sialophonin, leukocyte adhesion the CD11 integrin cluster, micro bacterial cell adhesion and the interleukin-4 receptor, which is interesting, as IL-4-mediated functions are altered in IBD.

Because some of the genetic factors involved in Crohn's disease may also contribute to ulcerative colitis susceptibility, research into Crohn's disease may assist in further understanding both types of IBD.

The genetics of inflammatory bowel diseases (in brief)

The inflammatory bowel diseases (IBD) comprise complex genetic disorders with multiple contributing genes. Linkage studies have implicated several genomic regions as likely containing IBD susceptibility genes, with some observed uniquely in Crohn's disease (CD) or ulcerative colitis (UC) and others common to both disorders

The best replicated linkage region, IBD1 on chromosome 16q contains the CD susceptibility gene, and NOD2/CARD15 is expressed in peripheral blood monocytes and is structurally related to the plant R proteins, which mediate host resistance to microbial pathogens.

Three major coding region polymorphisms within NOD2/CARD15 have been highly associated with CD among patients of European descent having one copy of the risk alleles confers a 2-4-fold risk for developing CD whereas double-dose carriage increases the risk 20-40-fold.

All 3 major CD variants exhibit a deficit in NF-kappaB activation in response to bacterial components. Carriage of NOD2/CARD15 risk alleles is associated with ideal location, earlier disease onset, and structuring phenotype.

Other IBD genomic regions include IBD2 on chromosome 12q (observed more in UC), and IBD3, containing the major histocompatibility complex region. A short genomic region has been associated with CD on chromosome 5q but the precise contribution gene is as yet unidentified.

The characterization of additions IB susceptibility genes could potentially lead to the identification of novel therapeutic agents for IBD make possible a molecular reclassification of disease and increase understanding of the contribution of environmental factors (notably tobacco and the intestinal microbial milieu) to intestinal inflammation.

Comparison of ulcerative colitis and Crohn's disease

Feature	Ulcerative Colitis	Crohn's Disease
Distribution	Diffuse, Distal Predominance	Segmental Or Diffuse Often Proximal Predominance
Recturn	Always Involved	Often Spared
Microscopic Distribution	Diffuse	Often Spared
Depth Inflammation	Mucosal	Transmural
Sinus Tracts And Fistulae	Absent	Often Present
Structurea	Absent	Often Present
Granulomas	Absent	Often Present

IBD and Irritable bowel syndrome (IBS)

Many people confuse IBD and IBS. Both conditions tend to occur in young adulthood. However, IBD and IBS are very different conditions.Irritable bowel syndrome is commonly known as "spastic colon". IBS is also a chronic disorder of the gastrointestinal tract.

It is characterized by altered bowel habits (diarrhea and/or constipation) abdominal pain, bloating, gas and mucus in stool.The hallmark of IBS is that the abdominal pain is relieved with a bowel movement. Although the symptoms of IBS can be distressing, there is no bowel inflammation with IBS. All diagnostic studies will be normal with IBS.

What should we do if a person has IBD

Talk to the health care provider. If your child has IBD then health care provider will discuss the symptoms of IBD and physical examination will be done. She/he probably should go for a blood test to check for an inflammatory process or anemia.

X ray studies such as upper gastrointestinal series or barium enema may be necessary for diagnosis. An endoscopic procedure to exam the small or large bowel is essential. Samples of the intestinal lining called biopsies can be obtained during these tests.

Diagnosis

Family medical history will be checked. Child stool is checked by endoscope.Colonoscopy, upper endoscopy,biopsy and barium study of intestines.

Treatment

Anti-inflammatory drugs, immunosuppressive agents. Sometimes surgery may be suggested by doctor.Removal of colon and ileoanal anastomosis(ileoanal pull through)

Caring child suffering from IBD

Balanced diet with adequate calories. Encourage the child to eat small meals throughout the day.If child has symptoms like diarrhea,malnutrition,lose weight quickly,repeated bouts of diarrhea or abdominal cramping then contact doctor for IBD treatment.

Genetics of IBD

Epidemiological studies reveal a significant genetic contribution to the pathogenesis of IBD. Population based studies find that familial recurrence among patients with IBD is approximately 10%.

In addition, the relative risk to siblings of affected individuals (λs) is estimated to be 30-40 fold for genetic contribution in IBD. The concordance rate is significantly greater in monozytic than dizygotic twins for both CD (50-58% vs. 0-12%) and UC(6-14% vs. 0-5%).

Importantly, even though there are distinct phenotypic Either CD or UC are at increased risk developing either form of IBD. This suggests that although there are phenotype-specific susceptibility loci at least some genes will be shared by UC and CD.

Table 1. Summary of previously identified IBD susceptibility loci

Locus	Chromosomal Region	Comments	Variation Identified
IBD1	16q12	CD Specific CARD15 Gene	YES
IBD2	12p	Possibly UC Specific	NO
IBD3	6p	IBD HLA Region	Potential HLA Alleles
IBD4	14q11-12	Possibly CD Specific	No
IBD5	5q31	CD -Specific Cytokine	Yes
IBD6	19p13	Cluster	No
IBD7	1p36	IBD	No

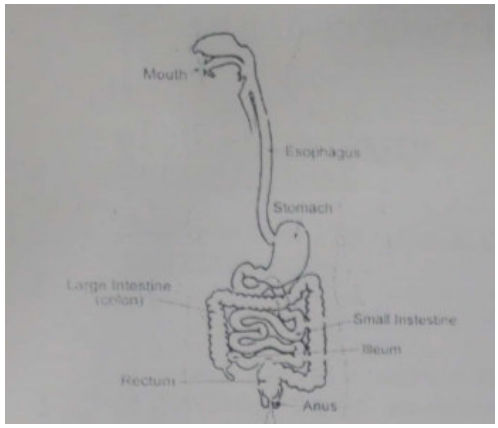
The first true replication of an IBD susceptibility locus (the IBD1 locus on chromosome 16, LOD score=5.79) came as a result of pooling genotype data from over 613 families collected by 12 centers distributed throughout North America, Europe and Australia.

Genome wide searches (GWS)

The two genome wide searches listed below involving greater than 220 families and the meta-analysis involving over 700 families clearly show that most IBD loci reported to date are common. This is not at all unexpected as most genetic variation is commonly found in all populations of the world.

Furthermore these results demonstrate the overall importance of the IBD loci located on chromosomes 3p and 6p. Despite the inherent difficulty in replicating linkage results in complex human traits, the results for the 3p and 6p loci are surprisingly consistent across multiple genome wide studies.

Genome wide search in Canadian families with inflammatory bowel disease reveals two novel susceptibility loci.



CD and UC are commonly classified as autoimmune diseases the prevalence of IBD increased in individuals with other autoimmune diseases particularly ankylosing spondylitis, psoriasis, sclerosing cholangitis and multiple sclerosis.

There is strong evidence from twin studies, familial risk data and segregation analysis the IBD especially CD is genetic. CD and UC are considered complex genetic traits as inheritance doesn't follow any simple mendelian model. IBD linked to chromosome 16p12-q13 (IBD1), 12p13 (IBD2) and 6p (IBD3)

SUMMARY

Mutations within the NOD2/CARD15 gene have recently been shown to be associated with crohn's disease. Investigated the prevalence of the three common NOD2/CARD15 mutations (Arg702Trp, Gly908Arg, and 3020insC) in 180 patients with crohn's disease, 70 patients with ulcerative colitis and 97 controls. In patients with crohn's disease,

prevalence of NOD2/CARD15 mutations were correlated to clinical and demo graphical parameters.

In crohn's disease patients, 35.6% carried at least one mutant allele of NOD2/CARD15 mutations compared with 14.3% of patients with ulcerative colitis (P=0.006) and to 15.5% of controls (P=0.0001). Genotype phenotype analysis revealed that NOD2/CARD15 mutations determined younger age at disease diagnosis (P=0.03), ileal disease location (P=0.01) and ileocecal resections (P=0.0002). Interestingly reoperation with resection of the anastomosis was significantly more frequent in patients with NOD2/CARD15 mutations (P=0.01).
Investigations support the current hypothesis that NOD2/CARD15 mutations are associated with a phenotype of crohn's disease with younger age at diagnosis, ileal involvement, ileocecal resections and a high risk of postoperative relapse and reoperations. NOD2/CARD15 mutations might therefore be used to identify high risk, patients for relapse prevention strategies.

The current working hypothesis for the pathogenesis of crohn's disease (CD) suggests that it is caused by a deregulated immune response towards antigens of faecal bacteria in a genetically susceptible host. Although several loci within the human genome have been linked to CD, the identification of mutations in the NOD2/CARD15 gene situated at chromosome 16q12 within the inflammatory bowel disease (IBD) 1 region and their association to cd has been a major step forward in understanding the disease pathophysiology.

CARD stands for Caspase recruitment domain containing protein.NOD stands for nucleotide binding oligomerization domain. The NOD2/CARD15protein encoded by NOD2/CARD15geneis an intracellular receptor for bacterial products that controls signals that lead to activation/inactivation of nuclear factor kappaB(NFKB). NFKB in turn activates tumor necrosis factor alpha(TNFa) which activates inflammation response.
NOD2 senses muramyl di peptide derived from peptidoglycans of bacterial cell walls.
TLR2--.>NFKB-->TNFa-->INFLAMMATION
TLR4-->NFKB-->TNFa-->INFLAMMATION

Mutations in NOD2/CARD15 prevents communication and leads to lining of the gut to be inflammed.

NOD2/CARD15 is known to act as an intracellular receptor in monocytes for bacterial components triggering activation of NFkappa-B and thus leading to subsequent activation of the inflammatory response. Within the NOD2/CARD15 gene, there are three independent mutations recently found to be associated with CD two missense mutations (Arg702Trp and Gly908Arg) and one frame shift mutation (3020insC).

The 3020insC mutation results in a truncated protein leading to an altered stimulation of NF-KappaB after bacterial activation although these mutations fit very well into the present pathogenetic hypothesis, they are only found in approximately 30% of CD patients. Therefore genotype phenotype correlations were conducted to find out if NOD2/CARD15 mutations are associated with a distinct clinical subtype of CD.

Mutations within the NOD2/CARD15 gene and their correlations to clinical data have been performed by others with differing results. Associations were found to ileal involvement fibrostenotic or fistulizing behavior and younger age at diagnosis, whereas other investigators failed to notice a relationship to the subgroups of the Vienna classification.

However, up to now no clinically relevant role for the NOD2/CARD15 mutations has been described. Thus, an accurate genotype/phenotype analysis is required to elucidate the role of mutations in the NOD2/CARD15 gene and to characterize in more detail their clinical contribution to the course of CD.

We therefore investigated the three common NOD2/CARD15 mutations (Arg702Trp, Gly908Arg, 3020insC) in 180 patients with CD and related the results to the demographic and disease phenotype.

Defining complex contributions of NOD2/CARD15 gene mutations and tobacco use on Crohn's disease phenotype

Multiple factors, particularly IBD family history, tobacco use leads NOD2 mutant genotype that influence Crohn's disease (CD) heterogeneity. They performed a multi-center retrospective record analysis of 275 unrelated patients with CD. Age at diagnosis, IBD family history, Jewish ethnicity, tobacco use at diagnosis, surgical history, disease site and clinical behaviors were correlated with genotype for NOD2 mutations, and all risk factors were

assessed for independent influence on outcomes of disease site, behavior and surgery free survival.

Genetics of IBD

Approximately 15% of patients with IBD have first degree relatives affected individuals is 8.9% for offspring. 8.8% for siblings, and 3.5% for parents. The incidence of the disorder among first-degree relatives of patients with IBD is 30-100 times that of the general population.

Moreover, although relatives of patients with crohn's disease are indeed more likely to have crohn's disease than ulcerative colitis have a higher among this group than it is in the general population. Additionally relatives of patients with ulcerative colitis have a higher incidence of both. There is a genetic basis for these diseases as well.

A study of unselected twins from a Swedish twin registry demonstrated that dizygotic (monidentical) twins have the same rate of concordance that would be expected for siblings, whereas monozygotic (identical) twins have higher rates of concordance for both diseases.

There is no reported case of monozygotic twins in which one twin had crohn's disease and the other had ulcerative colitis, thus suggesting that these disorders have a similar but not identical genetic background. There is no increased risk for IBD among spouses of individuals with the disorder.

Discovery of a gene linked to Crohn's disease

Scientists from the United States and Europe reported important findings from 2 independent studies that demonstrated a mutation in a gene known as NOD2, located on chromosome 16, that appears to be associated with Crohn's disease. The NOD2 gene encodes a protein associated with the innate immune system.

The NOD2 gene is found in monocytes that normally recognize bacteria and are then activated to destroy those bacteria. The NOD2 mutations found in patients with Crohn's disease lead to a disruption in monocyte activation, thereby making it much more difficult for the NOD2 protein to recognize and respond to bacterial lipopolysaccharide(LPS)

LPS is component of the bacterial cell wall that is found in many species of organisms. Because of this defect in the NOD2 gene in patients with crohn's disease and the resultant inability to recognize bacterial LPS the immune system appears to overreact, leading to uncontrolled inflammation and destruction of intestinal cells.

The initial work that led to discovery of the NOD2 gene and its association with this disorder involved the identification of NOD1 and NOD2 proteins as mammalian counterparts of plant disease resistant gene products that function as receptors within the cell (cytosolic receptors) for bacterial LPS.

Subsequently NOD2 was characterized as being highly restricted to monocytes, having the ability to induce nuclear factor kappaB (NF-KappaB) activation. NF-kappaB activation results in monocyte activation and a protective immune response.

Identification of the NOD2 gene association with Crohn's disease

Hugot and colleagues previously identified a susceptibility locus for crohn's disease on chromosome 16. Using positional cloning strategy, Hugot and coworkers then identified 3 NOD2 gene mutations that confer susceptibility to Crohn's disease by means of altering the recognition of bacterial LPS and or by activating NF-kappa B in monocytes. Truncated NOD2 protein associated with crohn's disease.

They also showed that the disease associated NOD2 variant was functionally less active in conferring responsiveness to bacterial LPS. These investigators hypothesized that a deficit in the ability to sense bacteria in monocytes could result in an exaggerated inflammatory response by the adaptive immune system.

Implications of the NOD2 gene discovery

There is clear evidence for the activation of intestinal lymphocytes, macrophages, and other cells of the immune system in leading to an unregulated immune response in IBD. In any immune response there is a specific antigen that serves as a trigger for the response and as a target for the effector arm of the response. Most of the antigens in the intestinal lumen are of microbial origin.

In IBD that antigenic trigger is most likely a common, nonpathogenic microbial agent within the intestine against which the patient mounts an activated immune response. In healthy individuals there is a finely tuned, low grade chronic inflammation in the intestinal lamina propria caused by intestinal bacteria.

Failure to suppress this inflammatory response due to mutations in the NOD2 gene, could result in the uncontrolled immune response with in the intestine in patients with IBD. As a result of failure of normal suppressor mechanisms, immune activation in IBD may be inappropriately vigorous and prolonged. Based on the NOD2 gene findings, the genetic basis of IBD may be due to a genetically determined inability to mount an appropriate immune response against bacterial LPS.

Therefore patients with IBD are genetically programmed to mount an intense immune response nonspecifically because of the compensatory response to common luminal bacteria or bacterial products. These luminal bacteria although they may have no role as etiologic agents are capable of triggering inflammatory immune responses if encountered by genetically programmed immune cells of the intestinal innate immune system.

It should be noted that the NOD2 gene on chromosome 16 is only 1 of a number of gene loci that have been implicated in chron's disease (4 to date). At least 5 additional loci (4 for crohn's disease and 1 for ulcerative colitis) have been implicated a possible chromosomal region in which genes that confer susceptibility to IBD may be identified in the future.

The NOD2 gene mutations that have been described to date are found in a subset of patients with chron's disease. In the future, additional genes involved in the altered recognition of bacterial cell wall products may also be implicated in the pathogenetic basis of IBD,

Tumor necrosis factor and the pathogenesis of IBD

The inflammatory response in IBD is redundant, complex and not completely characterized. Several investigators have hypothesized that the complexity of the inflammatory cascade argues against the expectation that immuno therapy can ever be sufficient to control disease activity in patients with crohn's disease.

However laboratory and clinical research suggests that tumor necrosis factor (TNF) serves as a pivotal inflammatory mediator and thus specific inhibition of this molecule interrupts major mechanisms of mucosal inflammation in crohn's disease.

TNF has numerous biologic properties involving inflammation, proliferation, and differentiation. TNF is also known to have a central role in shock due to sepsis, and in wasting syndromes due to a variety of cancers. In IBD, the proinflammatory properties of TNF are important.TNF leads to the activation of macrophages, the priming of neutrophils, and an increase in intestinal epithelial permeability. TNF is primarily a product of activated macrophages, though it is also produced by lymphocytes and natural killer cells.

Immunologic tissue injury results from the induction of proteases, prostaglandins, leukotriene's, eicosanoids, and other products.

However, some of these products (such as prostaglandin E_2) may also directly cause diarrhea by prompting mucosal secretion of chloride and potassium. Signal transducer and activator of transcription 6 (STAT6) is a key transcription factor involved in interleukin 4 (1L-4) and 1L-13-mediated Th2 response. The STAT6 gene is located on chromosome 12q13.3 14.1 (IBD region) and is therefore a positional and functional candidate gene for study in inflammatory bowel disease.

We investigated the G2964A polymorphism in the STAT6 gene was genotyped in 141 unrelated dutch Caucasian patients with ulcerative colitis, 183 patients with Crohn's disease and 173 healthy individuals by PCR and the amplification – created restriction Vienna classification and the patients with ulcerative colitis were classified with the age at onset, extent of disease and colectomy.

We did not find significant differences in genotype and allele frequencies of the G2964A polymorphism in the STAT6 gene between ulcerative colitis, crohn's disease and healthy controls. Subgroups of the patients with crohn's disease classified according to the Vienna classification and those with ulcerative colitis classified according to age of onset disease extension and colectomy did not differ in the distribution of this polymorphism. The STAT6 G2964A gene polymorphism is not involved in the overall susceptibility or in determining the phenotype of IBD.

TNF alpha and 11.10 SNPs act together to predict disease behaviour in crohn's disease

The cytokines tumour necrosis factor (TNF) and interleukin (IL) have been implicated in the pathogenesis of crohn's disease (CD) with increased concentrations reported in patients with active disease. However, limited data exist on their effects

on disease phenotype in the same population. Certain single nucleotide polymorphisms (SNPs) within the promoter region of the IL 10 (-1082G/A, -592C/A) and TNF (-308G/A, -857C/T) genes have been associated with altered levels of circulating IL10

TNF

An Australian based case-control study (304 CD patients 231 healthy controls of these four SNPs. Further investigation of two SNPs was conducted using a logistic regression analysis.

A possible association of both IL10 SNPs and TNF -857 with CD. Further investigation of relationship with disease severity showed a significant association of higher producing IL 10-1082G and TNF -857C alleles with structuring behavior, which was strongest when these alleles were combined and persisted after multivariate analysis (p=0.007 odds ration OR 2.37.95% CI 1.26 to 4.43) in addition the TNF -857CC genotype was independently associated with familial CD (p=0.03 OR 3.12:95% CI 1.15 to 8.46).

These two SNPs may help to predict disease behavior in CD patients, which may be clinically useful in shaping treatments of the disease at an earlier stage.

CARD4/ NOD1 is not involved in inflammatory bowel disease

Inflammatory bowel diseases (IBD) including crohn's disease (CD) and ulcerative colitis (UC) are complex genetic disorders, CARD15/NOD2 a member of the ced4 superfamily which includes Apaf-1 and CARD4/NOD1, has recently been associated with genetic predisposition to CD but additional genetic factors remain to be identified. Because CARD4/NOD1 shares many structural and functional similarities with CARD15 we tested its putative role in IBD

The 11 exons of CARD4 were screened for the presence of vitamins in 63 unrelated IBD patients. The only non-private genetic variation encoding for a substitution in the pesticide chain was genotyped in 381 IBD families (235 CD, 58 UC,81 mixed, and seven indeterminate colitis families) using a polymerase chain reaction – restriction fragment length polymorphism procedure. Genotyping data were analyzed by the transmission disequilibrium test.

Five of nine sequence variations identified in the coding sequence of the gene encoded for non-conservative changes (E266K, D372N, R705Q, T787M, and T787K). four were present in only one family. The remaining variant (E266K), which exhibited and allele frequency of 0.28, was not associated with CD, UC, or IBD.

Furthermore IBD patients carrying sequence variations in their CARD4 gene had a similar phenotype to those with a normal sequence. Results suggest that CARD4 does not play a major role in genetic susceptibility to IBD.As shown these genotyping methods have proved in our experience to be robust, easy to perform and cost effective.

Mutation And Genotyping Method	**Specific Primers**	**Sice of The PCR**
R702W:allelespecific.ARMS	SNP-specific primers : forwarded CACACTTAGCCTTGATG reverse wild type ATCTGAGAAGGCCCTGCTCC reverse mutated ATCTGAGAAGGCCCTGCTCC constant primers Forwarded TGAGGCAAAACAACTGAGAGC reverse GCAGACATTGATTTTACACAG	439 specific 102 control

PCR reaction componentsto identify any product fragments which are of this appropriate molecular weight.

ARMS PCR

Polymerase chain reaction is to permit rapid analysis of any known mutating in genomic DNA. Amplification refractory mutation system (ARMS) allows us to genotype solely the reaction mixtures after gel electrophoresis.

This is simple reliable and non-isotopic, which will clearly distinguish heterozygotes at a locus from homozygotes for either allele. It does not require any restriction enzyme digestion, allele specific primers are used one is for the mutant allele and one for normal allele. The primers differ from each other in their terminal 3 nucleotide.

Under proper annealing temperature and polymerase chain reaction conditions, these primers are directed to their complementary allele. Two complementary reaction takes place one is for normal allele and other is for mutant allele. Genotyping is based on whether there is amplification in one or both reaction.

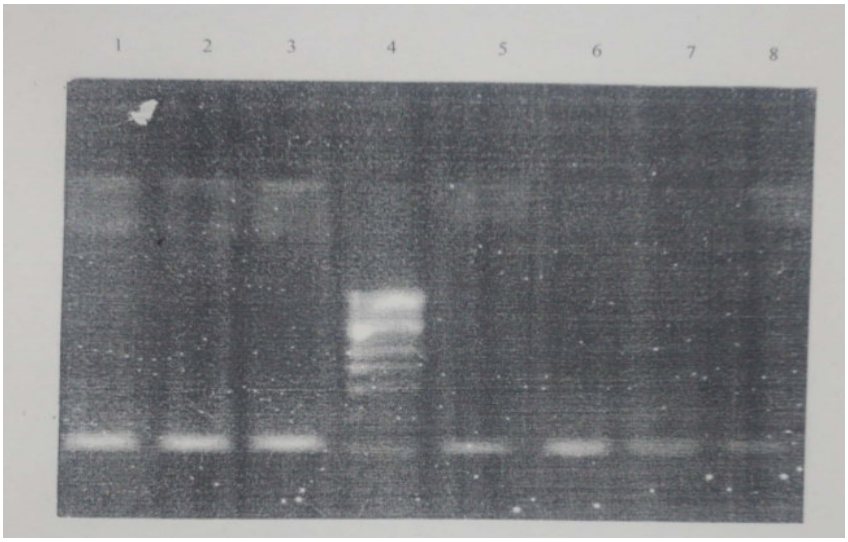
This system is able to discriminate all allelic variations both transitions and trans version mutations. So this system has a very good potential for diagnosing genetic disease, carrier screening. HLA typing human gene mapping, forensics and an allele specific PCR assay (ARMS).

Taq polymerase

A heat stable enzyme that adds the deoxynucleotides to the DNA template.

Primers

A primer is a short segment of nucleotides which is complementary to a section of the DNA which is to be amplified in the PCR reaction. Primers are annealed to the denatured DNA template to provide an initiation site for the elongation of the new DNA molecule.



LANES 1-3 and LANES 5-8 – patients samples showing 102 bp product indicating no mutation in the samples
LANE 4 – 100BP DNA ladder

RESULTS

The study includes blood samples of 20 patients suffering from IBD. The clinical data of all patients were collected the 3 mutations R702W.G908R and 100f sins are seemed to be more involve compared to other mutations.

In this study DNA was isolated from the patients and was subjected to ARMS PCR. The presence of 102 base pair product indicates no mutations and 439 base pair product indicates presence of mutation in the DNA..All of the 20 samples showed no mutation.

DISCUSSION

The study has been taken up to screen for the R 702 W mutation in the IBD patients. Most of the studies reveal that the presence of R702W, G908 R and 1007sins mutations as such cannot act as the principle cause of the disease.

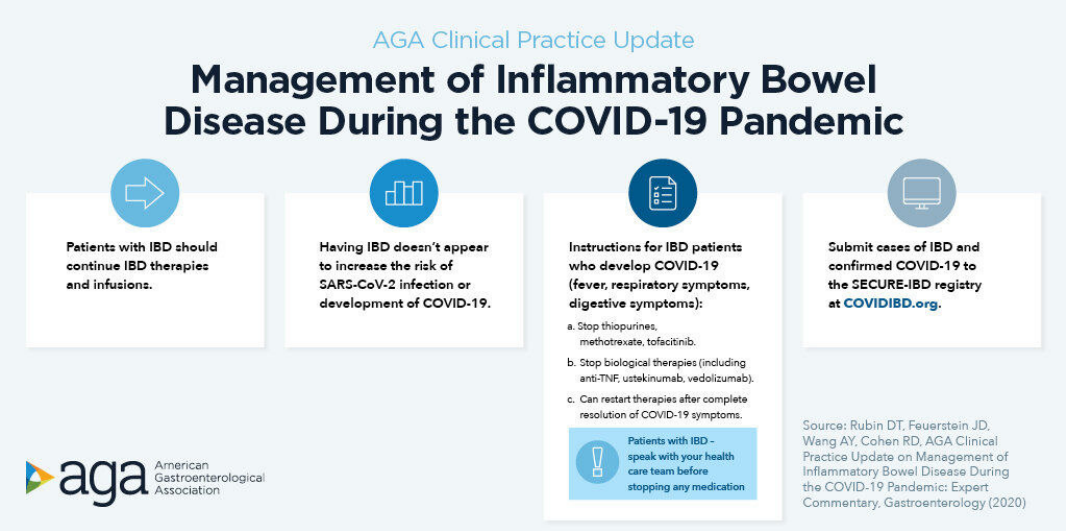
However the presence of these mutations acts as a potential risk factor towards the disorder. A thorough study has to be taken up in reference to the mutations in CARD 15 gene to arrive into a substantial conclusion as to what extent these mutations can contribute towards IBD

CONCLUSION

20 IBD patients were taken up for the study. Genomic DNA was isolated from blood using EDTA as anticoagulant. R702 W mutation was analyzed by ARMS PCR in 20 samples. None of the samples showed mutation. However other mutation in with the phenotypic manifestations of the disease and also to arrive at a definite conclusion upon the frequency of the mutations.

SCOPE OF FURTHER WORK

This study has been taken up for the R702W in the IBD patients. Most of the studies reveal that the presence of R702W, G908R and 100fsins mutations acts as an adding cause towards crohn's disease. But it should be noted that these mutations as such cannot act as the principle cause of the disease.



Currently only the CARD15 gene (majorly three variants) has been identified. Just one variant is not enough to conclude on onset of IBD. There need to be further work on other variants, auto immunity and infections agents in the population to come a conclusion as which variant or risk factor with susceptible genes is more common in producing IBD.

Further research can be listed into four priority area

- Identification of the IBD genes.
- A second priority is identification of casual genetic variation in understudied populations.
- Very little is known about the role of IBD genes in biology and pathophysiology.
- Research objective is a greater understanding of the influence of genetic variation on disease.

REFERENCES

[1]. https://www.medicinenet.com/script/main/art.asp?articlekey=41964
[2]. http://www.crohnsdisease.00home.com/tcells.html
[3]. https://www.homoeotimes.com/jan06/html/psoriasis-theme.htm
[4]. Denise K Bonen, Judy H Cho,The genetics of inflammatory bowel disease**,***Gastroenterology 124 (2), 2003, 521-36*
[5]. https://litairian.com/ibs-irritable-bowel-syndrome/
[6]. Teri A. Manolio, Francis S. Collins, Nancy J. Cox, et all. Finding the missing heritability of complex diseases .Nature. 461(7265), 2009, 747–753.
[7]. Sanjay Shete Christopher I.Amos, Testing for Genetic Linkage in Families by a Variance-Components Approach in the Presence of Genomic Imprinting,AJHG70(3), 2002, 751-757.
[8]. Institute of Medicine (US) Committee on Assessing Interactions Among Social, Behavioral, and Genetic Factors in Health; Hernandez LM, Blazer DG, editors.
[9]. Washington (DC): National Academies Press (US); 2006.Chang-Qing Zheng, Gang-Zheng Hu, Zhao-ShuZeng, Lian-Jie Lin, Progress in searching for susceptibility gene for inflammatory bowel disease by positional cloning, World J Gastroenterol. 9(8), 2003, 1646–1656.
[10]. C Büning, J Genschel, S Bühner, S Krüger, K Kling,,Mutations in the NOD2/CARD15 gene in Crohn's disease are associated ,with ileocecal resection and are a risk factor for reoperation, Aliment PharmacolTher19(10), 2004, 1073-8.