A COMPREHENSIVE EXAMINATION OF INFLAMMATORY BOWEL DISEASE FOR GENETIC COUNSELORS

by

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ABSTRACT

Inflammatory Bowel Disease is a complex, non-Mendelian auto-inflammatory/auto-immune disorder with increasing incidence worldwide. The pathogenesis of this disease remains elusive; however, the current theory postulates that the inflammation of the bowel is caused by an aberrant immune response to commensal gut bacterial. It is believed that this abnormal response occurs in a genetically susceptible host after exposure to environmental triggers. Research has still yet to prove a universal trigger for all cases of IBD

Currently the genotype-phenotype data is too limited to provide any kind of substantial diagnostic genetic test and relative risk data provides as 1 in 10-20 risk for first-degree family members. Roughly 20-25% of disease heritability is explained and researchers believe an additional portion of the "missing heritability" may be found in either risk alleles with small effect sizes or the non-coding regions of the genome affecting gene expression through gene-to-gene interactions.

Studies have shown patients are open and receptive to genetic counseling services; however, today's data provides counselors with little useful information but does not rule out the need for genetic counseling services. In the future a panel of many small effect genes may help to elucidate those at the greatest risk for disease.

The ability to determine those at the greatest risk for disease is of public health importance due to the ever-increasing burden of disease. With the average age of onset between 15-30 years old, the majority of an individual's working life may be inhibited by disease related complications reducing their productivity, as well as, increasing the time and cost for which disease related health care services are required. Together these factors and more create a burden of disease on both the individual and the public health community.

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PREFACE

This thesis comes one year after the onset of my IBD symptoms and since then my life has not been the same. It wasn't until I was hospitalized in January 2014 that I was given the diagnosis of ulcerative colitis. I wrote this not just as a graduate student or a researcher but also as a patient trying to understand why this happened to me. This was not an easy journey for me, and some potholes were significantly larger than others; however, I made it.

Thank you to my parents for their unconditional love and support. Thank you Ryan for so many things but especially for cross-country flights, multiple trips to the hospital, for telling me to keep going and to "just send it". Thank you to my girls, to the best friends anyone could ask for, the one thing that made the last two years worth it.

Thank you to Dr. Binion for finally getting me well. Thank you Dr. Shaffer for all his edits. Thank you to Dr. Finegold who taught me not just about the inner workings of life, but also about how life works. Sometimes, people are potholes, but that should never stop me from driving.

1.0 INTRODUCTION

Inflammatory Bowel Disease (IBD) can be clinically defined, in the most simple of terms, as a chronic inflammation of the gastrointestinal tract. The majority of IBD individuals fall into two diagnostic categories: Crohn's Disease (CD) or Ulcerative Colitis (UC). In regards to histology and the location of inflammation the two disorders are unique. However, in their ability to negatively affect the quality of an individual's life they are the same.

IBD is known for its alternating periods of inactive and active inflammation known as remissions and relapses or flares (Ananthakrishnan 2013; Conrad et al. 2014; Thompson et al. 2011; Ordas et al. 2012). An active flare state is when a person with IBD is actively experiencing symptoms which can consists of: general feelings of being ill, diarrhea, rectal bleeding, bloody stools, urgent bowel movements, frequent bowel movements, constipation, abdominal cramps and pain, fever, fatigue, or weight loss.

Flares can range in their severity and presentation depending both on the individual and the type of IBD. A mild or moderate UC flare may consist of mild diarrhea and urgent bowel movements once or twice a day. A severe UC flare may cause extreme abdominal pain, 10 to 12 bowel movements in a few hours, with feelings of incomplete evacuation or tenesmus, along with blood and/or pus and/or mucous in the stool.

CD differs from UC in that it often affects the small intestine and the inflammation penetrates into the deeper layers of the gastrointestinal wall. This often leads to the formation of

fistulas, which are sores that extend through the GI wall to other organs. A severe CD flare may consist of both weight and appetite loss, anal fissures, severe pain after eating, tenesmus, multiple bowel movements in a day and bloody diarrhea.

In efforts to find a cure for this debilitating disease researchers are still attempting to understand what causes it. However the pathogenesis, the mechanism(s) of disease, and the etiology, the factors that instigate the onset of disease, are still being elucidated.

The current working model of IBD's pathogenesis, presents a complex disease in which a dysregulated mucosal immune response to intestinal microbial flora in a genetically susceptible host, triggered by as yet to be determined environmental factors (Zhang and Li 2014; Hanauer 2006).

When individuals harboring IBD risk alleles are exposed to certain environmental triggers, their ability to correctly respond to commensal and pathogenic microbes in the gut is compromised. This altered microbial reaction, results in an improper immune response that sets off an unregulated and ill controlled inflammatory cascade. Currently, a large portion of the risk loci being investigated are linked to infection susceptibility, host-microbial response, and show overlap with other autoimmune diseases further strengthening the concept that IBD is an intricate communication error between the bacterial of the gut and the intestinal immune system (Ananthakrishnan 2013). Risk alleles have been found in genes that are associated with innate immune pathways (i.e. Th17 cells, CARD9, NOD2, reactive oxygen species and IL-1β*)* (Knights et al 2013).

While IBD is considered an auto-inflammatory disease it also shares the largest number of risk loci with the autoimmune disease diabetes type I; as well as, a significant number of loci with other auto-immune diseases such as: ankylosing spondylitis, an arthritis of the spine, and psoriasis,

which causes irritated, red, flaky patches of skin (Graham and Xavier 2013). These diseases share risk loci in pathways such as antigen presentation and intestinal mucosal homeostasis (Knights et al. 2013).

A significant amount of research has been spent investigating the link between genetic mutations in the intestinal immune system and the composition of the microbiome, particularly at the onset of disease. Questioning whether a change in the homeostasis of the commensal bacteria, possibly an increase in a particular type of bacteria triggers this aggressive immune response (Knights et al. 2013; Jostins et al. 2012; Xavier and Podolsky 2007; Ananthakrishnan 2013). Another theory is that host tolerance is somehow lost to normal gut microbes, causing harmless microbes to be inadvertently labeled as pathogenic and triggering an unnecessary immune response (Hanauer 2006).

Researchers have cast a wide net in an attempt to discern environmental triggers that precede the onset of IBD, particularly in conjunction with their connection to the microbiome. There is evidence, that prior gastrointestinal infections, as well as exposure to antibiotics, especially before the age of one, may increase risk of developing IBD. However, not all environmental triggers uniformly affect all forms of IBD. For example, while cigarette exposure acts as a risk factor for CD, it alternately acts as a protective factor for UC, or while having an appendectomy has been associated as a protective factor for UC, it has no effect on the development of CD. Diet and lifestyle have also been investigated with some research suggesting that diets high in fat and low in soluble fiber are associated with increased risk for the development of IBD. Additionally, it is believed that geographic location may play a role as there is some association with a north south gradient, with a greater incidence of IBD linked to those individuals living in more northern areas, where a relative decrease in UV exposure may connect these

individuals to the finding that those with IBD tend to be vitamin D deficient (Mouli and Ananthakrishnan 2014; Ng et al 2013).

While some individuals who suffer from IBD are able to identify their own personal triggers, such as particular foods or activities and can reduce their symptoms by avoiding these, there is no consensus on which environmental triggers are universal, as studies have only produced ambiguity. Without knowing the factors that precipitate IBD or the underlying pathology it is difficult to enact preventative measures to decrease the risk for disease in susceptible individuals. Therefore medicine must currently focus on treatments that target symptoms.

Current treatment modalities do not result in universal remission and many have serious side effects. Therapy is determined based on disease type, severity and location. Anti-inflammatories, steroids, and antibiotics are usually the first lines of defense for mild to moderate disease. In cases of severe disease immunosuppressive medications may be used, as well as two types of biologic therapy: anti-TNF agents which block the protein tumor necrosis factor alpha, i.e. infliximab, adalimumab, and anti-integrins used to block integrins, which are proteins used by white blood cells to travel to areas of active inflammation, such as the intestine, i.e. natalizumab. If the inflammation cannot be controlled, surgical removal of the affected bowel is another treatment option with CD patients up to 3 times more likely than UC patients to require surgery (Hanauer 2006).

While removal of the bowel is not ideal, the improvement in quality of life can be substantial. Life after surgery is a common topic among members of Internet-based support groups. The “Crohn’s and Colitis UK” Facebook group is just one of the many online support resources for those with IBD. It is an open forum to discuss all aspects of life with IBD, and many members use it to share their surgery experiences. “Get Your Belly Out” is an online movement

where individuals who have colostomy bags proudly post photos displaying their bags or surgical scares and share their stories with followers. The sharing of trials, tribulations, and treatment successes with others helps to improve confidence and tear down some of the stigma of life after surgery.

Without an identified cause(s) it is difficult for individuals to control the timing and duration of flares. The two most disruptive symptoms are pain and frequent, urgent bowel movements; which can severely affect the quality of every day life.

IBD can occur at any age; however, it often strikes individuals during their early formative years, the major peak of onset occurring between 15 and 30 years old , meaning most individuals affected by IBD suffer for more than two thirds of their lifespan (Hanauer 2006; Ordas et al. 2012; Molodecky et al. 2012). An earlier onset also inhibits individuals during their most productive years, diminishing their capacity to be active, productive members of society (Ng et al. 2013).

Boonen et al. (2002) studied Dutch IBD patients and their presence and ability to participate in the labor force. They found the labor force participation of IBD individuals was 6.5% lower than controls and the amount of chronic work disability was 17.1% higher, with CD patients being more affected than UC patients. Boonen et al. (2002) also adjusted for age, gender, and education, and found the odds ratio (OR) of chronic work disability in UC relative to controls was 2.6 (95% CI: 1.8–3.6) and 5.4 (95% CI: 3.7–7.8) in CD. Additionally, IBD individuals had higher numbers for disease-related sick days than controls and also often had lower numbers of non-disease-related sick leave compared to controls. Boonen et al. (2002) suggests that those with IBD are aware of the need to utilize sick days for disease related illness and so may consciously choose to work through minor illness to a lot for that fact.

Because of current detection and treatment modalities, IBD does not increase mortality to the point of decreasing the expected lifespan, resulting in an increased burden of disease not only for the individual but the community at large (Hanauer 2006*;* Ordas et al. 2012).

The Crohn's & Colitis Federation of America (CCFA) website, when accessed in April 2014 states that 1 in 200 Americans suffers from IBD. Hanauer (2006) discussed the burden of disease in the US, stating, IBD patients generate 700,000 physician visits, and 100,000 hospitalizations with 119,000 IBD patients considered disabled by their disease, annually. This data was taken from the National Institute of Diabetes and Digestive and Kidney Diseases epidemiological impact report from 1994. As the incidence of this disease has increased since then, about 30,000 new cases per year, these numbers are most likely an underestimate of the burden of disease today. Loftus (2007) stated that the likely prevalence of IBD in the US was somewhere between 1 million and 1.5 million. The cost of medications, endoscopies, colonoscopies, and hospital admissions can be staggering. According to the Centers for Disease Control website, www.CDC.gov, accessed in April of 2014, the overall health care cost for IBD is $1.7 billion. In 2008 the National Institute of Diabetes and Digestive and Kidney Disease investigated the burden of prescription medications associated with IBD, Table 1. below presents that information. However, the data is limited by the following: not all medications used for the treatment of IBD were investigated and the data was collected from retail pharmacies only.

Table 1. 2004 IBD Prescription Data

Data from 2004	UC	CD	Total
Number of Prescriptions	2,181,622	1,878,368	4,059,990
Retail Cost	$272,893,060	$261,504,663	$534,397,723

This table displays the number of prescriptions written and the totally cost of those prescriptions by disease type in the US in 2004. The table was adapted from the 2008 report from the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. The data is limited as is does not include all medications used to treat IBD and was collected from retail pharmacies only.

The incidence of IBD is steadily increasing around the world, with a current worldwide prevalence of 2.5 million people (Dinwiddie et al 2013). IBD is most commonly found in North America and Europe, but has also been seen in Israel, Australia, and South Africa. IBD, more so UC, is becoming more prevalent in places like Japan, South Korea, Singapore, northern India, and Latin America where previously it was a rare occurrence (Loftus 2004; Ng et al. 2013).

Many researchers have discovered a positive correlation between an increase in an "industrialized" or "westernized" lifestyle, occurring in the areas previously mentioned, and the incidence of IBD. This finding supports the role of environmental factors in the development of IBD (Ordas et al. 2012).

Because environmental factors alone do not appear to cause IBD deeper investigation into the genetics of IBD is warranted. Familial clustering and monozygotic concordance rates in twin studies support the existence of a genetic component. Recent advances in Genome Wide Association studies (GWAS) and Immunochip studies (specifically designed SNP chip arrays) have significantly increased our understanding of the possible genes and pathways involved in IBD. The number of risk loci currently stands at 163, associated with 300 known genes, however, these loci currently explain 20-25% of disease heritability (Jostins et al. 2012). The remaining "missing heritability" is postulated to be laying in wait: 1. in SNPs with only modest evidence of

association or 2. in gene-to-gene interactions (Duerr 2007; Zhang and Li 2014). Lee et al. (2011) suggested the missing heritability may be a result of small effect sizes that fail to reach GWAS significance levels, which complements Duerr. Lee et al. (2011) also suggests that causal variants may be missed due to lack of association caused by insufficient LD with SNPs on commercial arrays. In conclusion Lee et al. (2011) suspects a proportion of heritability will always be missing as a result of rare casual variants of small effect.

Between 2008 and 2013, 38 Genome Wide Association studies submitted to the Genome.gov database, have investigated the genetic component of IBD, CD or UC. The findings of these studies have reinforced the theory that IBD is a non-Mendelian, multifactorial disease requiring environmental trigger(s) to initiate the onset in a genetically susceptible host.

GWAS studies have aided in making significant progress towards identifying the pathogenesis of IBD. The genes identified through GWAS are linked to at least 7 different pathways, however how dysregulation of gene function and ultimately disruption in these pathways causes the IBD phenotype is still unclear (Dinwiddie et al. 2013).

When one combines the following factors: an increase in incidence of disease worldwide, an increasing burden of disease, an unknown etiology and unidentified heritability, it becomes quiet clear, IBD is quickly emerging as a major world public health concern.

This literature review first aims to bring together the most current epidemiological and clinical information pertaining to IBD, specifically Crohn's Disease and Ulcerative Colitis. A second aim is to gather information on environmental risk factors and other influences associated with IBD. A third aim of this review is to discuss the previous genetic research and what information has been gleaned from those studies. And the final aim of this paper is to summarize this information as it pertains to genetic counseling.

2.0 INFLAMMATORY BOWEL DISEASE

Inflammatory Bowel Disease (IBD) is a chronic inflammation of the gastrointestinal tract with recurring periods of relapse and remission. Periods of relapse, known as flares, can be mild to severe and intermittent or chronic. Currently there is no known pathogenesis, etiology or cure.

The majority of IBD cases can be divided into two major sub classifications: Ulcerative Colitis (UC) and Crohn's Disease (CD). There is also a third class of IBD called "indeterminate colitis" (IC) which represents about 10-15% of affected individuals (Sura et al. 2014). Rare forms of IBD include collagenous colitis, lymphocytic colitis, diversion colitis, and Behcet's disease; however, these will not be discussed in this paper.

It is medically necessary to appropriately diagnosis a patient for the purposes of effective medical management. For example, in severe cases where surgery is required, an ileal pouch-anal anastomosis is contraindicated when the diagnosis is CD (Geoboes et al. 2008). Early use of biologics is recommended to reduce the complications of CD, but is not the case for UC (Sura et al. 2014).

In the following sections we will discuss the clinical manifestations, histological presentation, and unique characteristics of CD, UC, IC, as well as childhood and adolescent onset forms of IBD.

2.1.1 Crohn's Disease (CD)

2.1.1.1 Clinical Features

Crohn's Disease (CD) is clinically recognized by the following symptoms: persistent diarrhea, rectal bleeding, urgent need to move bowels, abdominal cramps and pain, sensations of incomplete evacuation (tenesmus) and constipation, which can lead to bowel obstruction. Individuals with CD can also experience complications such as: abscesses, fistulas and strictures (Zhang and Li 2014). Fatigue, loss of appetite, weight loss, night sweats, and for women loss of a normal menstrual cycle are all common outward signs of disease (CCFA 2014). Almost 50% of individuals with CD experience extraintestinal manifestations such as: arthritis, uveitis, and erythema nodosum. Onset can occur at any age; however, it usually occurs between the ages of 15-30 years old (Laass et al. 2014).

2.1.1.2 Histology

CD can affect any part of the GI tract from the mouth to the anus and the inflammation commonly presents in a discontinuous fashion; where patches of the GI tract appear normal in between areas of severe inflammation (Laass et al. 2014).The common histological features are: non-cryptolytic granulomas, focal or patchy lamina proprial chronic inflammation, focal or anatomically discontinuous crypt distortion, ileal involvement, absence of features of ulcerative colitis, decreasing proximal to distal gradient of changes, and a normal mucosal surface (Feakins 2014; Margo et al. 2013).

2.1.1.3 Therapy

Treatments for CD can include: antibiotics, aminosalicylates, corticosteroids, immunomodulators, anti-TNF-antibodies and other novel biological drugs. Treatment choice is usually based of disease severity, location, and behavior and whether or not surgery is being considered or has already been performed. Exclusive enteral nutrition is recommended in children with acute disease as it provides the greatest gains for weight and growth with the least amount of side effects (Laass et al. 2014).

2.1.2 Ulcerative Colitis (UC)

UC can be further disseminated in to 3 sub categories based on location/extent of inflammation. First is proctitis: inflammation is limited to the rectum, second is left-sided colitis: inflammation extends from the rectum to splenic flexure, and third is pan-colitis: inflammation of the entire colon.

Table 2. is a modified table taken from Conrad et al. (2014) which presents the prevalence of some of the more common signs and symptoms of UC that can occur.

Table 2. Distribution of Signs and Symptoms in Adult Onset Ulcerative Colitis

Signs and Symptoms	Prevalence in Adult onset UC
Diarrhea	70 – 90%
Abdominal Pain	30 – 70%
Weight Loss	35 – 45%
Rectal Bleeding	50 – 90%
Extraintestinal Manifestations	2 – 15%

The data for this table was adapted from Conrad et al. (2014) and represents the prevalence of specific symptoms at the onset of UC in adult patients.

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2.1.2.1 Clinical Features

The physical symptoms of UC consist of: blood in the stool, increased bowel movement frequency, tenesmus, diarrhea, and abdominal pain (Ordas et al. 2012; Conrad et al. 2014). Individuals with UC may also experience: fatigue, loss of appetite, weight loss, night sweats, and for women loss of a normal menstrual cycle (CCFA 2014).

While most likely an underestimation, at least 1/3 of individuals with IBD have some type of extra intestinal manifestation. These include but are not limited to: arthritis, uveitis (inflammation of the covering of the eye), skin lesions and sacroilitis (inflammation of the large joints of the tail bone and pelvis) (Cummings and Rubin 2006).

Individuals with UC can commonly have other manifestations of disease such as inflammatory arthropathies and primary sclerosing cholangitis (PSC), which are the most common. PSC, an inflammation of the ducts of the liver occurs in about 2-10% of patients and occurs independent of IBD. Other extraintestinal manifestations include: erythema nodosum, pyoderma gangrenosum, episcleritis, uveitis and osteoporosis (Conrad et al. 2014; Cummings and Rubin 2006).

The onset of UC can occur at any age; however, the majority of individuals with UC will typically present between the ages of 15-30 years, with a smaller major group presenting between the ages of 50-70 years (Ordas et al. 2012; Hanauer 2006).

2.1.2.2 Histology

UC is distinct from CD in that the inflammation is contained to the mucosal surface and found only in the colon, always including the rectum, with a few exceptions. Conrad et al. (2014) states that nonspecific mucosal inflammation in the terminal ileum or “backwash ileitis” occurs in 10–

20% of UC patients and rectal sparing may occur in 30% of untreated children, 13% of adults with fulminant colitis or 44% of patients receiving topical or systemic treatment.

UC is characterized by its continuous pattern of inflammation along the epithelial lining with a visibly sharp transition from inflamed and normal mucosa, neutrophilic mucosal infiltrate, goblet cell depletion, 'cryptitis' and prominent crypt abscesses (Thompson and Lees 2011).

2.1.2.3 Therapy

In cases of mild to moderate UC, topical or oral aminosalicylates are used, e.g. mesalamine or sulfasalazine. Topical is most often used for patients with proctitis or left sided colitis. For individuals with moderate to severe disease that shows no response to aminosalicylates, steroids are the next approach, commonly prednisolone or an equivalent (Conrad et al. 2014).

In more severe cases where the individual is chronic and/or steroid dependent, immunosuppressive treatment is recommended with azathioprine or 6-mercaptopurine. Anti-TNF-alpha antibodies may be an option to induce and maintain remission in patients with steroid refractory or dependent disease (Conrad et al. 2014). A common surgical treatment is total proctocolectomy with ileal pouch-anal anastomosis, which may be required in patients with longstanding disease, in fulminant colitis, or in the prevention of colon cancer (Conrad et al. 2014).

2.1.3 Indeterminate Colitis

For some patients, approximately 10-15%, it can be difficult to give a diagnosis of UC or CD (Sura et al. 2014). An accurate diagnosis is critical for appropriate medical management as some therapies for the treatment of UC are contraindicated for CD and vice versa.

For individuals who clearly present with symptoms of IBD, but have ambiguous histological findings, they are given a temporary diagnosis of indeterminate colitis (IC) (Geoboes et al. 2008; Margo et al. 2013). Indeterminate colitis (IC) has also been described in the literatures as “uncertain colitis”, “inflammatory bowel disease unclassified (IBDU)”, “CIBD-unclassified” and “chronic idiopathic inflammatory bowel disease NOS” (not otherwise specified) (Margo et al. 2013).

2.1.3.1 Clinical Features

There are no exact clinical features for IC, other than an incongruent mix of CD and UC symptoms and histology. It is thought that a case presenting as IC may in fact be the early stages of UC, as most of these cases behave similar to UC and are later reclassified (Geoboes et al. 2008). IC is often the case in children, about 23% of new onset cases, particularly in those > 12 years old (Margo et al. 2013).

2.1.3.2 Histology

The characteristic histology of an individual with IC is extensive ulcerations with involvement of transverse and right colon, with the appearance of diffuse disease (Margo et al. 2013). Both severe mucosal and wall involvement is seen with non-aggregated transmural inflammation. There is often a discontinuous pattern with fissures reaching the muscularis propria and sharp transitions from ulcerated areas to adjacent normal mucosa (Margo et al. 2013).

2.1.4 Genetic Counseling Take Away Points

- The average age of onset for IBD is between 15 – 30 years old
- CD
    - Inflammation can affect any part of the GI tract and occurs in patches and penetrates deep into the epithelial layers.
    - Fistulas, strictures, and abscesses are common complications
- UC
    - Inflammation almost always includes the rectum and is usually confined to the colon with a few cases of ileum involvement.
    - Inflammation occurs in a continuous fashion along the mucosal surface.
- IC
    - 10-15% of IBD cases are considered “indeterminate”
    - Histological findings are an incongruent mix of both CD and UC features
    - It is important that other causes of inflammation such as bacterial infection are ruled out before the diagnosis is given
    - Correct diagnosis is important for proper medical management

2.2 CHILDHOOD AND ADOLESCENT IBD

Twenty-five percent of individuals with IBD are children or adolescents (Levine et al. 2011; Laass et al. 2014). And 20 to 30% have onset of symptoms before the age of 18, with diagnosis often occurring later (Conrad et al. 2014). 2% of diagnoses are in children less than 10 years old

(Cummings and Rubin 2006). CD tends to be more prevalent in pediatric cases compared to adult cases, which have higher rates of UC (Ruel et al. 2014).

Childhood onset can be further classified into 3 sub groups based on age at diagnosis: Infantile onset, diagnosis prior to age 1; Very early onset (VEO-IBD), diagnosis prior to age 6; and Early onset (EO-IBD), diagnosis prior to age 18 (Dinwiddie et al. 2013).

An interesting finding that separates childhood onset from adult cases is the clinical presentation of disease. Knights et al. (2013) explains that between the ages of 0 to 2 presentation is atypical with CD cases having affected colons and UC cases presenting with pancolitis (Knights et al. 2013). Another atypical finding mentioned by Laass et al. (2014) is that in adult onset CD, disease localization usually remains constant within the first year, however in 40% of children with CD the disease extends within the GI tract within the first 2 years of onset (Laass et al. 2014).

Table 3. Adult vs. Childhood Onset of Ulcerative Colitis

UC Classification	Prevalence in Adult onset	Prevalence in Childhood onset
Proctiitis	40 - 50 %	1.4%
Left-sided Colitis	30 - 40%	16%
Pancolitis	25 - 30%	82%

The information in this table was adapted from Conrad et al. (2014) and presents the difference in the percentage of cases that occur in each sub category of ulcerative colitis based on age of onset.

Graham and Xavier (2013) suggest that because we see both pediatric and adult cases of IBD, distinct environmental influences must exist that contribute to disease initiation rather than disease progression (Graham and Xavier 2013). However, research has uncovered age specific mutations, which in cases of VEO-IBD, are believed to play a more pivotal role. A few of the genes associated with early onset are: NOD2, IL-10RA, IL10-RB, IL-10, XIAP, ADAM17,

NCF2/RAC2 and NCF4, TNFRSF6B, PSMG1, SLC22A4/5 and an early-onset IBD panel, consisting of over 30 genes, is offered by Emory Laboratories. IBD researchers question whether an increased load of susceptibility genes or mutations in specific genes are what separates adult from childhood onset IBD. Evidence supporting the specific gene theory is the case of autosomal recessive loss of function mutations in IL-10 receptor (IL10RA and IL10RB) subunit genes, which have been documented to cause a severe CD phenotype without any apparent environmental triggers (Rogler 2011; Thompson and Lees 2011; Dinwiddie et al. 2013; Ruel 2014).

2.2.1 Genetic Counseling Take Away Points

- CD is more common in male children.
- Symptoms and disease progression are different between adult and pediatric cases with the majority of pediatric cases of UC diagnosed as pancolitis and the majority of CD cases diagnosed with colonic inflammation.
- Current research suggests that there is a greater genetic effect in childhood onset cases. Emory laboratories offers a panel for early onset cases.

2.3 SEX EFFECT

Kappelman et al. (2013) study of commercially insured Americans below the age of 65 found sex differences were only significant for CD. Conrad et al. (2014) also discusses a lack of significant differences in the appearance of UC between males and females.

Ruel et al. (2014) questioned whether the age of onset affected the male to female ratio of disease. The study found UC pediatric cases are about equal in the distribution of disease between males and females, however, adult onset cases tend to have a higher female prevalence of UC. Typically there are more males affected with CD in pediatric cases compared to adult cases where females with CD are more prevalent. This change in distribution appears to occur between the ages of 14-17 years, usually soon after puberty. This shift has also been seen in type 1 Diabetes, other immune-mediated inflammatory disease. It's possible a change in hormones may play a role in disease pathogenesis.

In Nguyen et al. (2006) study of racial and ethnic differences among adult IBD cases, and found AA females to have a slight predominance compared to females of the white and Hispanic cohorts, 63% to 51% and 45%, respectively.

2.4 INCIDENCE AND PREVALENCE

2.4.1 USA

According to the Centers for Disease Control (CDC) website, accessed in April of 2014, the current estimated prevalence of IBD in the USA is 1.4 million people. The CDC also estimates the incidence rates of IBD worldwide as follows: UC: .5 – 24.5 / 100,000 persons and CD: .1 – 16 / 100,000 persons.

Kappelman et al. (2013) utilized the 2009 US census data to estimate the prevalence of CD and UC for the USA. The investigators standardized the data for age, gender and region, estimating roughly 1,171,000 Americans have IBD with an approximately equal split between UC

and CD (593,000 UC and 565,000 CD). Of those affected roughly 5% or 62,000 were pediatric cases under the age of 20 years. (23,000 UC and 38,000 CD). The value of this data is limited as it only captures individuals who had commercial insurance and were younger than 65 years old. Because of this limitation the numbers are most likely an underestimate of true disease prevalence in the USA today.

2.4.2 Worldwide

Dinwiddie et al. (2013) reported that 2.5 million people worldwide have IBD. Molodecky et al. (2012) used a systematic review to establish changes in the incidence and prevalence rates of IBD worldwide analyzing 246 studies between 1950-2010. Their findings are provided in Table 4.

Table 4. Incidence and Prevalence Rates for IBD Worldwide

Incidence			
	Europe	North America	Asia and Middle East
UC	24.3 per 100,000 persons	19.2 per 100,000 persons	6.3 per 100,000 persons
CD	12.7 per 100,000 persons	20.2 per 100,000 persons	5.0 per 100,000 persons
Prevalence			
	Europe	North America	Asia and Middle East
UC	505 per 100,000 persons	249 per 100,000 persons	N/A
CD	322 per 100,000 persons	319 per 100,000 persons	N/A

Data for this table was taken from Molodecky et al. 2012 and represents the known incidence rates of IBD as of 2009 in Europe, Asia/Middle East, and North America and the prevalence rates of IBD in Europe and North America as of 2009.

After a time-trend analysis Molodecky et al. (2012) found that in CD vs. UC studies incidence rates increased with a statistical significance of 75% vs 60% respectively. Overall they concluded that IBD is an emerging global disease and more epidemiological data is needed to show accurate incidence and prevalence data across the globe.

3.0 ENVIRONMENTAL INFLUENCE AND RISK FACTORS

3.1 "WESTERNIZATION" THEORY

The "Westernization Theory" is an intriguing trend emerging in the worldwide epidemiology of IBD postulating that certain lifestyle changes and environmental exposures could put "westernized" individuals at greater risk for developing IBD.

The theory is based on the rate of increasing occurrence of UC and CD in parts of the world where previously it was very rare (i.e. Asia, Japan, Africa, and the Middle East) with the highest rates of growing incidence occurring in the developing or industrializing regions of these countries (Molodecky et al. 2012; Chen et al. 2014). As a result of these once rural populations becoming urbanized, there is a tendency to develop a more "Westernized lifestyle", which constitutes a higher socioeconomic status, an increase in stress, an increase in smoking, a change of diet to one higher in sugar, fat, and processed foods as well as greater access to medications such as antibiotics and NSAIDs (Ananthakrishnan 2013).

Additionally, in urban settings not only is access to medications more likely, but access to physicians aware of diseases such as UC and CD increases, resulting in a increased UC and CD diagnosis reporting in medical records (Molodecky et al. 2012; Chen et al. 2014; Danese and Fiocchi 2011; Ordas et al. 2012). In addition to the individualized or societal environmental factors, industrialization affected environmental factors such as increasing levels of atmospheric air pollution; specifically high levels of NO_2 and SO_2 have been shown to correlate to an increased risk of CD and UC (Zhang and Li 2014).

3.2 HYGIENE HYPOTHESIS

Along with a more westernized lifestyle individuals living in cities often live a more sanitary existence as well. The "hygiene hypothesis" postulates that reduced exposure to enteric infections during childhood inhibits the maturation of the mucosal immune system, which subsequently puts them at risk for inappropriate response to pathogens later in life (Ordas et al. 2012). Additional risk factors associated with this hypothesis are: helminthic infections and antibiotic use during childhood; breastfeeding; family size and/or sibship; urban vs. rural lifestyle; and personal and domestic hygiene in childhood.

Helminths are parasitic worms transmitted through soil contaminated with human feces harboring the worm eggs. Ruel et al. (2014) discusses that exposure to helminthes induces a strong type 2 T helper cell (TH2) and regulatory T cell (TREG) response. An exaggerated TH2 response is what is believed to drive the aberrant inflammation seen in UC. Also the TH2/TREG response is believed to oppose the type 1 T helper cell (TH1) response associated with CD (Ruel et al. 2014; Ordas et al. 2012). According to this evidence perhaps, a lack of exposure to this type of parasite early in life leaves the immune system ill practiced in these pathways, so that later in life, when these pathways are activated by some other trigger, the immune system responds more robustly.

3.3 IMMIGRATION EFFECT

Additionally, improved hygiene might be linked to an "Immigration Effect". Several studies have found that there is an increased risk to individuals and their offspring who emigrate from areas of low IBD prevalence to areas of high IBD prevalence. Cummings and Rubin (2006) cited a 1999

study that stated when populations move from low risk areas, i.e. rural settings, to higher risk areas, i.e. developing cities, the incidence of IBD increases in those populations. Conrad et al. (2014) cited a similar finding and attempted to link the increased risk back to the hygiene hypothesis, suggesting that certain exposures during infancy/early childhood are involved in the development of IBD, specifically the risk for UC (Chen et al. 2014; Conrad et al. 2014).

3.4 CIGARETTE SMOKING

One of the most well studied risk factors is cigarette smoking. Studies have shown a 2 fold increased risk for development of CD in individuals who smoke. However, smoking appears to be a protective factor against the development of UC. It has also been observed that when cessation of smoking occurs the protective factor also ceases and individuals will see an increase in disease activity. A meta-analysis, cited by Ordas et al. (2012) showed that smoking is protective against ulcerative colitis compared with non-smoking (odds ratio [OR] 0·58, 95% CI 0·45–0·75) (Ordas et al. 2012; Zhang and Li 2014). Nguyen et al. (2006) also found the role of smoking to be consistent between Hispanic and white cohorts stating in their results that the Hispanic UC patients had higher rates of nonsmoking at time of diagnosis associated with more extensive disease and higher rates of colectomy.

Ananthakrishnan (2013) proposes that smoking, in connection to the composition of the microbiome, has modifying effects on the intestinal immune response possibly caused by free radical-mediated oxidative stress. However, researches and doctors do not promote smoking as a condoned behavior to reduce the risk of UC.

3.5 APPENDECTOMY

Appendectomy is another well-investigated risk factor that appears to reduce the risk of developing UC. Appendectomies that occur before the age of 20 have been shown to reduce the risk of developing UC by 69% (Ordas et al. 2012). However, it has not been shown to be protective against the development of CD. Whether this association is of a true causality is still yet to be established (Ananthakrishnan 2013).

3.6 VITAMIN D

Data is emerging that links vitamin D to having immunological functions within the innate and adaptive immune system, as well as, playing a role in various autoimmune and inflammatory diseases (Yin and Agrawal 2014; Zhang and Li 2014). Yin and Agrawal (2014) explain that vitamin D plays a role in mucosal barrier homeostasis, which is a critical component of IBD development. vitamin D has been seen to be associated with preservation of epithelia cell junction integrity, which aids in the proper regulation of the immune/inflammatory response, by reducing the risk of pathogen infiltration this in turn, reduces the risk of mucosal damage and IBD (Yin and Agrawal 2014).

Studies have shown a high prevalence of vitamin D deficiency in patients with IBD, (16–95% according to Mouli and Ananthakrishnan (2014)) and found that supplementation of vitamin D, specifically D_3, has a significant effect on the activity of IBD. Mouse models have shown increased levels of colonic inflammatory cytokines in vitamin D receptor knockout mice. However, genetic association studies are still inconclusive and larger randomized studies are still

needed to prove its effectiveness as a potential treatment (Yin and Agrawal 2014; Ananthakrishnan 2013; Mouli and Ananthakrishnan 2014).

Wang et al. (2014) performed a meta-analysis of research that investigated four polymorphisms of the vitamin D receptor gene, *VDR*, and its connection to IBD. The polymorphisms were labeled by the restriction enzymes: ApaI (rs7975232), TaqI (rs731236), and BsmI (rs1544410) and FokI (rs10735810). They concluded that in Caucasians especially, ApaI polymorphism may increase the risk of CD, and TaqI polymorphism may decrease the risk of UC.

3.7 MICROBIOME

The number of catalogued bacteria within the human gut current stands at roughly 1150 different bacterial species. However, each individual person hosts roughly 160 different bacterial species (Zhang and Li 2014). This suggests that not every human gut microbiome is the same and different permutations of bacteria may have varying affects on health. IBD researchers are now investigating whether particular compositions of bacterial species play a role in who does and does not develop IBD. Another question is whether or not certain environmental exposures can alter the microbiome from "homeostasis" to dysbiosis that triggers the immune response seen in IBD patients (Knights et al 2013).

3.7.1 Antibiotics

Use of antibiotics can upset the natural equilibrium of the microbiome by eliminating pathogenic and commensal bacteria, leaving the gut susceptible to an over abundance of certain types of

bacteria. Kostic et al. (2014) puts forth the idea of "colonization resistance" one of the many ways commensal bacteria keeps its end of the symbiotic bargain. Commensal bacteria occupy a sufficient amount of the gut surface area leaving pathogenic bacteria unable to find a "niche" in which to colonize and overwhelm the host. If this theory is true, a round of broad-spectrum antibiotics could make an individual susceptible to colonization of unwanted bacteria (Kostic et al. 2014). Reduced levels of beneficial bacteria such as: *as Bifidobacterium, Lactobacillus, Bacteroidetes* and *Firmicutes* along with increased levels of less helpful bacteria such as Enterobacteriaceae and Fusobacterium spp. have been found in individuals with IBD (Zhang and Li 2014). More specifically Enterobacteriaceae and pathogenic bacteria that promote inflammation are increased in patients with Crohn's disease and *Escherichia coli* are increased in patients with UC (Ruel et al. 2014; Zhang and Li 2014). Pediatric cases appear to have increased levels of aerobic and facultative-anaerobic bacteria, whereas adult IBD is associated with an increase in numbers of anaerobic bacteria (Ruel et al. 2014).

3.7.2 Bacterial Infections

Both Ordas et al. (2012) and Ananthakrishnan (2013) agree that episodes of prior gastrointestinal infections may lead to chronic IBD, going on to explain that exposures, in a genetically susceptible host, may change the gut flora enough to trigger the chronic inflammatory response that is IBD. Ordas et al. (2012) discusses specific exposures to *Salmonella*, *Shigella*, and *Campylobacter* bacteria, doubles the risk for the development of UC. And Ananthakrishnan (2013) discusses that the infection risk is bidirectional; infections prior to disease onset may be a trigger that aids the onset of disease; and then post IBD onset, the individual then becomes susceptible to enteric infections, particularly *Clostridium difficile*.

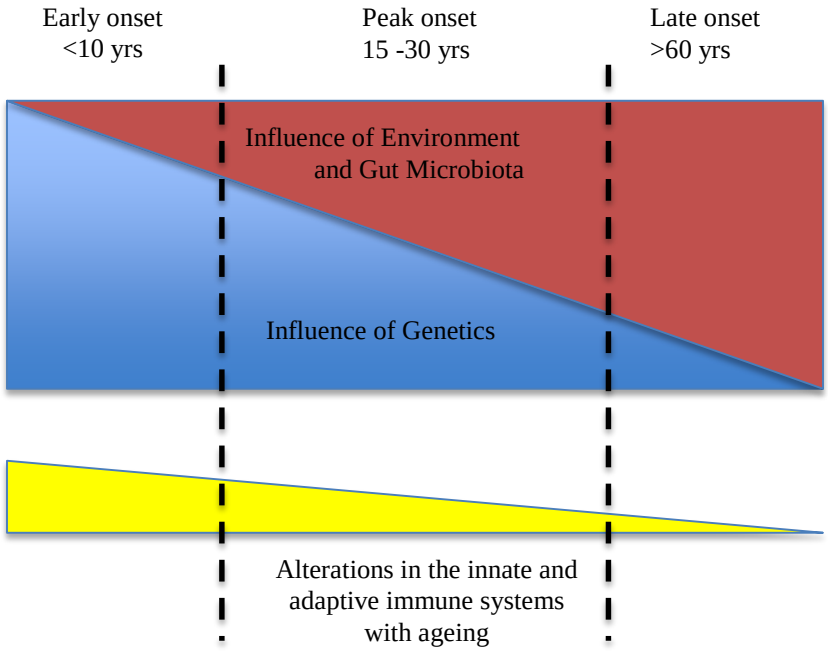
In 2013, A.N. Ananthakrishnan, gives an interesting commentary, in the Journal of Pediatrics, on a paper written by Kronman et al. in 2012 called “Antibiotic exposure and IBD development among children: a population-based cohort study”. The authors concluded that, in childhood, antianerobic antibiotic exposure is associated with IBD development. Ananthakrishnan’s commentary expresses that because a bacterial infection is often tied to exposure to antibiotics it is difficult to determine which exposure is linked to the underlying etiology of IBD. However, Ananthakrishnan (2013) agrees that the literature strongly supports antibiotic exposure and IBD risk. While the risk is low it should still bring physicians to pause before prescribing antibiotics at an early age.

3.7.3 Breastfeeding

The microbiome of the gut develops soon after birth and historically a child’s first exposure to food outside the womb was breast milk. With the advent of baby formula many children are never exposed to breast milk leading researchers to question whether a link exists between breastfeeding and IBD development (Ruel et al. 2014). Specifically with UC, Ordas et al. (2012) states breastfeeding is protective when the duration is for more than 3 months. Ruel et al. (2014) discusses the difference in bacterial diversity between breastfed and formula fed babies, stating breastfed babies have mostly helpful bacteria while formula fed babies have a more diverse bacterial load that includes more pathogenic types. The extent that formula or breast milk effects risk requires further investigation.

3.7.4 Microbiome integrity and age

The biodiversity of the gut goes through age specific transitions which correlate to the 3 peak periods of disease onset: early onset occurring <10 years, peak onset 15 – 30 years, and elderly or late onset occurring >60 years. The microbiota of the gut alters its diversity and is unstable during these transitions. After birth the microbiome does not reach full stability and strong diversity until adulthood. It is influenced by type of delivery, vaginal or C-section, diet, breast milk or formula, and then again when a switch to solid foods is made. The stability of the bacteria of the gut is also influenced by illness and puberty. Stability is often maintained until after the age of 60 where the microbiome is more susceptible to fluctuations and distress (Kostic et al. 2014; Ruel et al. 2014). The immune system also differs as we age. In the elderly, immunosenescence occurs resulting in increased risk for infection and autoimmunity to occur. Age is an integral component in identifying the degree to which certain factors contribute to the onset of IBD. Figure 1. demonstrates how the effects of genetics appear to have a stronger influence earlier in life, conversely environmental influences appear to have a greater impact on IBD development in the elderly.



The above figure was adapted from Ruel, J. et al. (2014) and demonstrates the differing affects of factors contributing to IBD over the lifetime.
Figure 1. Affects of Age on IBD

This figure also demonstrates how genetics plays a greater role in the development of childhood IBD. Recessive IL-10RA and IL-10RB loss of function mutations are capable of causing early onset Crohn's phenotype without an environmental trigger (Ruel et al. 2014; Dinwiddie et al. 2013).

3.8 MEDICATIONS

Other types of medications have been studied as possible disease modifiers. Research on NSAIDs usage has been shown to disrupt the integrity of the intestinal mucosal barrier, which can make the gut lining more susceptibility to pathogenic bacteria, in turn potentially igniting an inflammatory

response (Ruel et al. 2014). And if the NSAID user also has genetic susceptibility to IBD they are at an increased risk for a dysregulated response. While there have been several studies that show exposure to antibiotics and NSAIDs are increased in individuals who later develop IBD, the exact risk still needs to be validated (Ananthakrishnan 2013; Ordas et al. 2012; Zhang and Li 2014).

3.9 HORMONAL INFLUENCE

Researchers have also questioned whether there is a hormonal influence associated with onset of IBD. Studies have shown elevated risks for the development of IBD in women who use oral contraceptives as well as women who have endometriosis. (Ananthakrishnan 2013 10; Ordas et al. 2012).

3.10 PSYCHOLOGICAL STRESS, ANXIETY AND DEPRESSION

While Ordas et al. (2012) did not find any data to support the role of psychological stress on the development of UC, Ananthakrishnan (2013) discusses that psychological changes can have an effect of the autonomic and sympathetic nervous system, which can be influential in the activation of the immune system. Rogler (2011) explains that while stress does not directly cause the manifestations of IBD it acts as a disease modulator. However, there is no strong human data to rule in or rule out the etiological role of an individual's mental state and the development of IBD (Zhang and Li 2014).

3.11 DIET

Diet is certainly on the list of factors that need be more heavily investigated; however, prospective and even retrospective case / control studies are very difficult to perform and even more difficult to infer causality. Most often diet studies require patient journaling and backfilling is a common occurrence, which limits the integrity of the results. However, as Ananthakrishnan (2013) explains patients commonly inquire about dietary options that may reduce symptoms without the cost and side effects of pharmaceuticals.

Studies have attempted to find a connection between diet and disease, by examining the pre-illness diet of those with IBD. Common trends seen in IBD diet studies are a reduction of fiber specifically soluble fiber from fruits and vegetables and an increased intake in high fat in individuals with IBD (Ananthakrishnan 2013). Specific types of fats were found to have varying affects on IBD and that information is provided in Table 5. below.

Table 5. Dietary Components as Potential Risk Factors

	Arachidonic acid	Linoleic acid	Dietary n-3 polyunsaturated fatty acid	Docosahexaenoic acid	Carbohydrates	Soluble fiber	Animal Protein
Risk of UC	Increased	Increased	Protective	Protective	Inconsistent	N/A	N/A
Risk of CD	N/A	N/A	N/A	N/A	Inconsistent	Protective	Increased

The above table was created using data from Ananthakrishnan, A.N. (2013). This table presents the differing affects certain foods have on IBD phenotype.

Knights et al. (2013) suggests that long-term dietary habits can affect the gut microbiome and that shifts in particular nutrients, such as sulfur and tryptophan could start a chain of events that ends in the triggering of an inflammatory response. Graham and Xavier (2013) also suggest tryptophan as well as dietary levels of salt can be upstream triggers of the inflammatory response.

However, survey data suggests food triggers amongst IBD patients are not universal and it has been proposed that particular triggers may be linked to specific genotypes. Rigorous studies are still needed to validate the utilization of diet as a therapy option (Ananthakrishnan 2013).

3.12 GENETIC COUNSELING TAKE AWAY POINTS

- Westernization Theory / Hygiene Hypothesis: There appears to be a correlation between IBD and living a “westernized lifestyle.
- Cigarette Smoking: Studies have shown smoking is a protective factor in cases of UC but increases the risk of CD.
- Appendectomy: Studies have shown appendectomy is a protective factor in cases of UC but does not have an effect on CD
- Vitamin D: Vitamin D deficiency appears to be associated with IBD and vitamin D supplementation may be a possible adjunct therapy
- Microbiome: The integrity of gut bacteria is a major area of investigation. The exact factors (i.e. bacterial infections, antibiotics, NSAIDs, hormones, age) that change the gut bacteria and exactly what that change looks like are still being elucidated.
- Stress: Stress may act as a disease modulator affecting disease symptoms but not disease onset.
- Diet: No clear-cut associations between diet and disease have been validated.

4.0 GENETIC RESEARCH IN INFLAMMTORY BOWEL DISEASE

"IBD is a complex genetic disorder characterized by non-Mendelian inheritance, in complete penetrance and disease susceptibility but not disease certainty" (Konda et al. 2006).

4.1 FAMILY STUDIES

A landmark article in IBD family history research is the Orholm et al. (1991) paper; which reported that a 10-fold increase in familial risk strongly suggests a genetic cause. His study surveyed 637 patients with IBD in the county of Copenhagen, Denmark. These individuals were followed for 9 years to determine the rates at which their relatives developed IBD. The study findings are represented in Table 6. below.

Table 6. First-Degree Relative Risk for Developing IBD

	Risk for 1st degree relative to develop UC	Risk for 1st degree relative to develop CD
Proband has UC	9.5 fold greater	1.8 fold greater
Proband has CD	4.4 fold greater	10.3 fold greater

The above table was created using data from Orholm et al. (1991) and demonstrates the greater risk to develop the same disease type as first degree family members and that CD appears to confer the greatest overall risk.

Orholm et al. (1993) utilized the same data to analyze the mode of inheritance through complex segregation analysis, concluding there is a dominant gene in effect in 10% of UC patients, and in 7% of CD patients there is a recessive gene at play.

Russell and Satsangi (2004) explains in "IBD: A Family Affair", that the risk is highest for siblings of a CD proband and may be as great as 1 in 3 but that in general first-degree relatives of a proband with IBD have a 1 in 10-20 risk (Russell and Satsangi 2004). Ruel et al. (2014) discusses that there appears to be a greater prevalence of positive family history in cases of pediatric onset, suggesting genetic factors may play a more substantial role in these cases.

4.1.1 Anticipation

Researchers began to investigate the possibility of anticipation as a cause for familial IBD when studies showed children of IBD affected parents often had an earlier age of onset. Earlier studies supported the existence of anticipation (Grandbastien et al. 1998; Lee and Lennard Jones 1996; Polito et al. 1996; Satsangi et al. 1998); however, more recent studies have found evidence that suggests anticipation is most likely an artifact of methodological biases.

Peeters et al. (2000) discusses several interesting points that may create the appearance of anticipation; first both parents and medical professionals are more aware of the signs and symptoms of IBD which may aid in earlier diagnosis also with each generation diagnostic accuracy improves. Methodological biases are also discussed; such as recall bias and preferential ascertainment of later onset parents leading to selection or truncation bias and finally inadequate follow-up may all contribute to the manifestation of anticipation.

A German study published in the year 2000 investigated the likelihood of anticipation in IBD. The study population consisted of 2,007 IBD patients with sporadic disease and 472 multiplex familial cases (including 103 affected parents and 99 children of affected patients). The results of the study found that the mean-age-of onset in children was 19.4 years earlier than their affected parent. When the investigators corrected for the confounding variables of higher age of the parental cohort and longer diagnostic interval in a general linear model there was no significant difference and no apparent anticipation ($P \geq 0.52$). The authors stipulate that ascertainment bias is the most likely explanation for the presentation of anticipation in the raw data and go on to state that the power to detect true anticipation in a complex disorder is quite low and conclude that the results of this study provide further evidence that anticipation in IBD does not occur (Hampe et al. 2000).

Russell and Satsangi (2004) reported evidence of a strong concordance between disease type and location in twin and multiplex family studies. However, stating that even though more than 75% of children with IBD were diagnosed younger than their IBD parent(s) the possibility of true genetic anticipation was unlikely.

4.1.2 Ethnicity

4.1.2.1 Ashkenazi Jews

Yang et al. (1993) investigated the potential ethnic predilection of IBD in individuals of Jewish decent. The results of that study concluded that the lifetime risk to develop IBD in first-degree relatives of non-Jewish probands who had UC was 1.6% or probands who had CD 5.2%. First-degree relatives of UC Jewish probands had a lifetime risk to develop IBD of 4.5% and 7.8% when

the proband had CD. (p value for comparison between Jews and non Jews: 0.028; between UC and CD: 0.005). Yang et al. (1993) goes on to explain that the differing risk values support the theory that IBD does not fit a single Mendelian gene model.

While IBD may not be a Mendelian disorder, evidence still supports the existence of a genetic component. Ordas et al. (2012) explains that overall individuals of Ashkenazi Jewish decent have a rate of UC 3 to 5 times that of other ethnicities and Yang et al. (1993) showed that incidence and prevalence was consistent when compared to other ethnic groups in the same location, and the consistency was perpetuated over different time periods and geographical locations.

4.1.2.2 African American and Hispanic

Nguyen et al. (2006) published research investigating the racial implications in adult IBD. The study consisted of 1,126 subjects: 830 White, 127 African American (AA), and 169 Hispanic. This large study allowed for significant power to detect any racial differences that may exist.

They found that the distribution of disease subtype, UC or CD, was consistent across all groups with a ratio of 3:2 (CD:UC) and the average age of diagnosis was similar in all races, 26.4 yr. Nguyen et al. (2006) investigated the degree to which family history occurred across these three groups first by examining the prevalence of IBD family history across all subjects which was reported at 25.6% (95% IC: 23-28%). Then prevalence of family history was broken down by race: white = 28.6%, AA = 18.1%, Hispanic 16.6%. Nguyen et al. (2006) believed that underlying genetic differences between racial groups and ascertainment bias could possibly explain the lower prevalence of family history among AA and Hispanics compared to the white cohort; however, the findings of this study were consistent with the findings of previous research in studies of children with IBD (Kugathasan et al. 2005; Eidelwein et al. 2005).

Next the researchers broke down family history based on diagnosis. In cases of UC there was no statistical difference between the racial groups. Conversely in cases of CD, the data showed white subjects were more likely to have a family history of IBD, 30.6%, compared to AA subjects, 19.8%. Hispanics had an even lower prevalence compared to white subjects, 17.9% vs. 30.6%, respectively. This study found that there are racial differences in IBD phenotype and that the underlying causal genes are most likely not universal either.

4.2 TWIN STUDIES

Twin studies are an integral part of genetic research. Twin studies that show increased concordance rates in monozygotic (MZ) compared to dizygotic twins (DZ) is evidence of an underlying genetic factor that is attributing to disease. IBD Twin studies have reported an elevated concordance amongst MZ twins, 6% - 15% for UC and 30% - 37% for CD. The larger percentage of concordance in CD twins compared to UC twins suggests there is a stronger genetic component underlying the onset of CD (Conrad et al. 2014; Russell and Satsangi 2004; Ordas et al. 2012; Brant 2011). Because concordance is not 100%, extraneous factors such as environmental exposures are still worth investigating in regards to the onset of IBD (Brant 2011; Conrad et al. 2014). Twin studies have also been used to investigate the effects of smoking and disease onset. One study found that amongst Swedish and Dutch IBD twin cohorts, smoking revealed a 5 fold protective effect in cases of UC discordant MZ twins (Brant 2011). The success of twin studies provided the confidence needed to take research to the next level, molecular genetics, in an attempt to find causal genes.

4.3 GENOME WIDE ASSOCIATION STUDIES

In cases of non-Mendelian disease, family and twin studies can only provide so much direction and clarity. Genome Wide Association studies (GWAS), utilizes registries of multiplex families and seek linkage that is greater than that expected by chance association (Konda et al. 2006). GWAS studies operate under the hypothesis 'common disease, common variant', meaning if a disease is common, then any associated risk alleles will appear in more than 1-5% of the population (Manolio et al. 2009). While GWAS is better at detecting weak associations compared to linkage studies it still cannot direct casual information; however, it has proven to be the most effective way to identify IBD candidate genes worth further investigation.

Jostins et al. (2012) article was a landmark meta-analysis of 15 GWA studies, from which the data was validated with a custom SNP array called the Immunochip. The Immunochip contains almost 200,000 SNPs and >700 small insertion–deletions identified by previous GWAS loci for 12 immune-mediated diseases—Crohn's disease, ulcerative colitis, coeliac disease, type 1 diabetes mellitus, psoriasis, ankylosing spondylitis, multiple sclerosis, rheumatoid arthritis, IgA deficiency, autoimmune thyroid disease, primary biliary cirrhosis and systemic lupus erythematosus. A total of 186 loci were studied using SNP information from the 1,000 Genomes project. However it should be noted that genetic variation of individuals of non-European origin is under-represented in this array (Van Limbergen, J., et al 2014).

The Immunochip gave Jostins et al. (2012) more power as it enlarged the sample size to included 40,000 IBD genomes for verification. And as a result allowed Jostins et al. (2012)

research to bring the total number of risk loci from 92 to 163. They also found 110 loci associated with both UC and CD, while 23 were UC specific and 30 were CD specific. Two risk loci for CD were protective against UC, PTPN22 and NOD2, which would suggest CD and UC have different biological mechanisms. The great degree of overlapping loci suggests nearly all biological mechanisms involved in one disease are also involved in some capacity in the other.

Previous GWA studies only covered relatively common genetic variants by utilizing a significance threshold of >1% frequency with in the population, which can fail to capture the variant with a rare allele frequency (Graham and Xavier 2013). Jostins et al. (2012) was able to identify candidate genes in 53% of the 163 loci, almost twice as many as previous studies. However, only 29 SNPs showed LD to variants in the 1000 Genomes Project data, which supports the theory that a majority of the genetics of IBD lays within non-coding regions of the genome. Variants in the noncoding regions tend to alter gene expression; therefore, gene expression may be a key to understanding the mechanisms underlying the onset of IBD (Graham and Xavier 2013). Also these risk variants of noncoding regions make functional interpretation challenging in turn making it difficult to identify genotypes that relate to particular pathway associated phenotypes (Kaser and Pasaniue 2014).

4.3.1 Parent-of-Origin Effect

Fransen et al. (2012) investigated the possibility of a parent-of-origin (POO) effect in the 2012 paper "Limited Evidence for Parent-of-Origin Effects in Inflammatory Bowel Disease Associated Loci." The authors believe that genomic imprinting could explain some of the "missing heritability" seen within IBD. The authors' reasoning for a possible POO effect is the observation that, children with CD mothers were at a higher risk for developing CD than were children of

fathers with CD and proposed that this finding could be explained by either inactivation of the paternal allele by genomic imprinting or the mother's diet or genotype could have an affect on the developing fetus, where maternal proteins or circulating RNA pass through the placenta and influence the fetal epigenome.

They looked for POO effects in two cohorts of IBD patients: one Dutch and one Indian. Subjects were genotyped using the iCHIP (Immunochip) through Illumina Inc., and combined the UC and CD trios and investigated the known 28 overlapping loci. The authors state they had significant power; however, their results were only "nominally significant" for genes IL12B and PRDM1 in the Dutch UC+CD cohort; NOD2 in the Dutch CD cohort; and IL10 in the Indian UC cohort. Findings could not be replicated outside of the cohort in which they were found. In the end the authors state that while POO effects could not be determined at this time, it is possible in the future, with better technology and larger family cohorts a POO effect may be discovered (Fransen et al. 2012).

4.4 GENETICS AND PATHOGENESIS

Thus far data from GWA studies has identified 163 loci associated with at least 300 known genes, linking back to roughly 56 biological pathways mainly related in some way to the immune system, especially the mucosal immune system and microbial interactions. (Jostins et al. 2012; Dinwiddie et al. 2013). Table 7. below shows a portion of identified IBD genes and their pathway involvement.

Table 7. IBD Genes Categorized by Disease Association and Pathway Involvement

IBD-related Processes	UC genes	CD genes	UC/CD genes	Total
T-cell regulation	IL2, TNFRFSFP, PIM3, IL7R, TNFSF8, IFNG, IL21	NDFIP1, TAGAP, IL2RA	TNFSF8, IL12B, IL23R, PRDM1, ICOSLG	15
Restitution	ERRFI1, HNF4A, PLA2G2A/E	STAT3	NKX2-3, RTEL1, PTGER4	9
Immune cell recruitment	IL8RA/IL8RB	CCL11/CCL2/CCL 7/CCL8, CCR6	MST1	8
Solute transport	AQP12A/B, SLC9A3, SLC26A3	SLC9A4, SLC22A5, SLC22A4	0	7
Innate mucosal defense	SLC11A1, FCGR2A/B	NOD2, ILTN1	CARD9, REL	7
Il-23/Th17	IL21	STAT3	IL23R, JAK2, TYK2, ICOSLG, TNFSF15	7
Immune Tolerance	IL1R1 / IL1R2	IL27, SBNO2, NOD2	CREM, IL10	7
Epithelial barrier	ECM1, HNF4A, CDH1, GNA12	MUC19, ITLN1	0	6
B-cell regulation	IL7R, IRF5	IL5, IKZFI, BACH2	0	5
Paneth cells	0	ITLN1, NOD2, ATG16L1	XBP1	4
Antigen presentation	0	ERAP2, LNPEP, DENND1B	0	3
Oxidative stress	HSPA6, DLD, PARK7	PRDX5, BACH2, ADO, GPX4, GPX1, SLC22A4, LRRK2, NOD2	UTS2, PEX13, CARD9	14
Autophagy	PARK7, DAP	ATG16L1, IRGM, NOD2, LRRK2	CUL2	7
Intracellular logistics	TTLL8, CEP72, TPPP	VAMP3, FGFR10P	KIF21B	6
Apoptosis/necroptosis	DAP	FASLG, THADA	PUS10, MST1	5
ER stress	SERINC3	CPEB4	ORMDL3, XBP1	4
Cell migration	ARPC2, LSP1, AAMP	0	0	3
Carbohydrate metabolism	0	GCKR	SLC2A4RG	3

The information for this table was adapted from Khor et al. (2011) and presents a portion of known IBD genes and their pathway and disease involvement.

One of the most significant findings is that genes involved in maintenance of the mucosal barrier tend to be UC specific while microbial recognition factors; specifically, autophagy, and innate immune system microbial recognition processing appear to be CD specific. This coincides with the histological findings that UC inflammation remains in the superficial layers of the mucosal epithelial, while CD inflammation is found in the deeper layers penetrating into the lumen (Danese and Fiocchi 2011; Parkes 2012).

Jostins et al. (2012) explains that 70% of the IBD loci are shared with other complex diseases, many of them, more than would be expected by chance, are associated with immune-mediated disease, including those of an auto-inflammatory nature (Jostins et al. 2012; Knights et al. 2013). Table 8. Below is a sample of the shared loci between autoimmune disorders and IBD.

Table 8. Genetic Overlap of Autoimmune Diseases and IBD

Disease Name	IBD genes	CD genes	UC genes	Total
Coeliac Disease	5	6	2	13
Type 1 Diabetes	4	5	1	10
Rheumatoid Arthritis	2	3	4	9
Systemic Lupus Erythematosus	3	1	2	6
Multiple Sclerosis	2	1	2	5
Type 2 Diabetes	1	2	2	5
Alopecia	0	2	2	4
Asthma	2	2	0	4
Vitiligo	0	4	0	4
Leprosy	1	2	0	3
Ankylosing Spondylitis	1	1	0	2
Behcet's	2	0	0	2
Atopic dermatitis	1	0	0	1

The information in this table is adapted from Lees, C.W., et al. (2011) and presents the number of IBD genes that overlap with other autoimmune diseases.

4.4.1 IBD and the Immune System

The exact understanding of the underlying pathogenesis involved in IBD is still under investigation. One suggested theory is that defects in the epithelial barrier allow an excessive number of microbes into the lamina propria triggering the immune response causing an over activation of the mechanisms used to mediate the immune/inflammatory response. In the following sections this paper will discuss a few areas of both the innate and adaptive immune systems and how dysregulation of certain components can be related back to the clinical findings of patients with IBD.

4.4.1.1 Defensins

Defensins are antimicrobial peptides with the ability to destroy Gram-negative and Gram-positive bacteria, fungi, yeasts, parasites and viruses. Defensins are part of the non-specific, innate immune response and therefore are indiscriminate in their attack. Two key factors allow defensins to attack pathogens and not host cells. First host cell membranes contain cholesterol and defensins appear to avoid attacking cells containing cholesterol. Second, pathogens contain specific external molecules that are unique to them and are not found on host cells called pathogen-associated molecules or pathogen-associated immuo-stimulants. When a defensin comes into contact with this type of molecule both the inflammatory response and phagocytosis are utilized to remove the pathogen. Phagocytosis utilizes neutrophils and macrophages to envelope and destroy the pathogen (Alberts et al. 2002). In colonic biopsy samples of UC patients expression of selected human beta-defensins are up-regulated. What exactly causes this increase in defensin production is still unclear (Ordas et al 2012).

4.4.1.2 Dendritic Cells

Dendritic cells are also part of the innate immune system and fill two roles: first removal of pathogens via phagocytosis and second acting as antigen-presenting cells used to activate T-cells of the adaptive immune system (Alberts et al. 2002). The number and stimulatory capacity of activated and mature dendritic cells are elevated in UC patients and circulating numbers of dendritic cells show a positive correlation to disease activity; suggesting these cells are part of the mechanism that causes inflammatory perpetuation in UC (Ordas et al. 2012). What perpetuates this activation of dendritic cells is still unclear.

4.4.1.3 Toll-like Receptors

Toll-like receptors (TLR) are found on the surface of macrophages, neutrophils and the lining of the lung and mucosal surface digestive tract. They are the body's alarm system and alter both the innate and adaptive immune systems. When a pathogen-associated immune-stimulant adheres to a TLR, signals are sent for phagocytosis to occur and changes in gene expression stimulate the innate immune response known as the complement cascade (Alberts et al. 2002).

TLR4 expression is extremely uncommon in healthy controls; however, TLR4 expression is substantially increased in lamina propria cells of patients with ulcerative colitis. Suggesting changes in TLR genes (i.e. TLR4 D299G polymorphism) can alter susceptibility to enteric infections or cause the host to lose tolerance to commensal bacteria triggering the adaptive immune response (Ordas et al. 2012).

4.4.1.4 NF-kB pathway

Not all foreign bodies that come into contact with the host are pathogenic; one example is food. A food allergy is when the body mistakes food for a pathogen and initiates the adaptive immune

response every time that food is eaten (Alberts et al. 2002). An autoimmune disease is when the adaptive immune response losses its ability to distinguish host cells from pathogen cells. In a person who does not have IBD it is hypothesized that commensal bacteria helps the immune system by down regulating the inflammatory genes, blocking the NF-kB pathway from being activated. This is what allows a person to not have an inflammatory response every time we come into contact with harmless bacteria. In a person with IBD exposure of commensal bacteria does trigger an inflammatory response and the NF-kB pathway is not inhibited and researchers are attempting to determine exactly how this occurs (Hanauer 2006). In a healthy individual, commensal bacteria turns off inflammatory genes by blocking ubiquitination and inhibiting the NF-kB pathway from progressing. When pathogenic bacteria bind to a toll-like receptor (TLR) on the cell surface inflammatory factors connected to the NF-kB pathway are generated and intermediate kinases are stimulated, leading to phosphorylation of the inhibitor of B kinase and subsequent dissociation of NF-kB protein which is then moved to the nucleus switching on inflammatory genes such as; interleukin receptor–associated kinase; myeloid differentiation factor toll/interleukin-1 receptor; then allowing ubiquitination to occur (Hanauer 2006). However, in an individual with IBD it is believed that this pathogenic response is some how activated by commensal bacteria which are abundant in the gut therefore resulting in a continuous and destructive response.

4.5 PHARMACOGENETICS

Attempts have been made to find pharmacogenetic factors that may translate to better medical management as a shift towards personalized medicine becomes more attainable. Currently

clinicians utilize TPMT (thiopurine methyl-transferase) genotyping or enzymatic analysis to determine the efficacy of using thiopurine drug therapy such as Azathioprine. There are two alleles associated with TPMT, H (high), and L (low), which present in an autosomal co-dominant inheritance pattern. Individuals who are H/H (wild type) have high enzyme activity and can metabolize full dose regimens of therapy. H/L heterozygotes have intermediate enzyme activity and should receive a reduced recommended dose. These individuals may be at risk for myelosupression. Finally, L/L homozygotes have low to no enzyme activity, recommended dosing may cause 6-MP cytotoxicity and these individuals are at the highest risk for life-threatening myelosuppression; therefore, thiopurine therapy is not recommended (Weinshilboum and Sladek 1980; Prometheus Therapeutics & Diagnostics 2014).

Variants of the MDR1, TNF and migration inhibitory factor genes have been linked to corticosteroid refractoriness or sensitivity in CD and UC. MDR1 has also been associated with a higher risk of cyclosporine failure in patients with steroid-resistant UC (Beaugerie and Sokol 2012). Response to Infliximab seems to vary between IL23R homozygous risk increasing and risk decreasing carriers with the homozygous risk increasing variants more likely to be treatment responders (Beaugerie and Sokol 2012).

4.6 IBD AND GENETIC COUNSELING

Genetic counselors want to be able to provide their patients with genetic testing options that can provide prognostic information, statistics about recurrence risks for future pregnancies and disease

susceptibility for unaffected family members. However in situations of non-Mendelian disease this information is either not accessible or lacks clinical utility.

4.6.1 Risk to Family Members

Russell and Satsangi (2004), published a well-researched review of 20 family studies ranging from 1980 to 2002 and 4 major European twin studies to determine the familial risks that exists for IBD. Russell and Satsangi (2004) found that factors that increased risk were: Ashkenazi Jewish ancestry, multiple affected family members, and the proband having CD. The general risk to a first-degree relative for developing IBD is 1 in 10-20 and siblings of IBD probands are at the highest risk for developing IBD, with greater risk being associated with CD probands. The relative risk for CD siblings is 25-42 and the relative risk for UC siblings is 8-15. Offspring of an IBD proband are considered to have the next highest risk after siblings, with parents of an IBD proband having the lowest risk. The age of the offspring in question also affects the relative risk as IBD general occurs between the ages of 15-30, the risk begins to decrease after the age of 30. When both parents have IBD, offspring have at least a 1 in 3 lifetime risk of IBD. For a monozygotic twin of a CD proband the lifetime risk is 1 in 3 for IBD and with a UC proband around 1 in 5.

In regards to family history CD probands have an affected first-degree relative with CD between 2-16% of the time and a relative with IBD 5-22% of the time. UC probands have an affected first degree relative with UC or IBD 5-15% of the time. Third degree relatives are still at an increased risk compared to the general population however it is considerably less than first-degree relatives (Russell and Satsangi 2004; Orholm et al. 1991; Yang et al. 1993).

4.6.2 NOD2

NOD2, one of the first susceptibility genes identified for CD, is one gene that can offer prognostic information for patients and families with a certain level of confidence, as such, for the purposes of this paper only NOD2 will be discussed in detail due to its current use in clinical testing.

NOD2, is involved in host signaling pathways and is associated with activation of nuclear factor (NF)-kB, a transcription factor involved in cellular inflammatory responses and macrophage apoptosis. Mutations in NOD2 reduce activation in (NF)-kB disrupting the development of commensal bacteria tolerance. Lack of NOD2 expression in intestinal epithelial cells, reduces the host's normal response to microbial byproducts, causing overgrowth of bacteria and a greater potential for a breakdown in the mucosal barrier. It is also suggested that NOD2 variants disrupt mucosal homeostasis by affecting the formation of regulatory and effector T cell responses (Hanauer 2006).

NOD2 is the most well studied IBD gene and is the only gene to have significant prognostic information (Cho and Brant 2011). NOD2 has been associated with ilieal involvement and a more aggressive clinical course of CD; i.e., higher risk of intestinal strictures, earlier need for first surgery and reduced postoperative disease-free interval (Beaugerie and Sokol 2012; Benitez et al. 2014). NOD2 has also been shown to be a protective factor against the development of UC (Van Limbergen et al. 2014).

The three major risk alleles for NOD 2 are: SNP 8 (R702W) [C2104T], SNP 12 (G908R) [G2722C] and SNP 13 (1007fs) [3020insC] (Prometheus Therapeutics & Diagnostics 2014). The prevalence of CD is about 20 in 10,000 (0.2%) in either Europe or North America and roughly 7 out of 20 will have NOD2 variants. Roughly 30% of CD cases from the Western world carry at least one of the three major risk alleles and individuals with at least one of those variants has a 3

to 4 fold increased risk to develop CD. Overall, 10% of individuals with a NOD2 variant will not have IBD (Rogler 2011).

Results of a NOD2 gene test may not accurately represent the risk to an individual who is not of a European ancestry as previous studies have found that NOD2 risk alleles are not universal across racial groups (Nguyen et al. 2006). In a study of 626 Japanese IBD patients and 292 controls PCR was used to determine the allele frequencies of the three common NOD2 variants (C2104T, G2722C, and 3020insC) found in white populations. None of the patients were carriers for any of the variants investigated (Inoue et al. 2002). Nguyen et al. (2006) explains that there are also significantly lower NOD2 allele frequencies in African Americans compared to white CD patients and unaffected white individuals.

4.6.3 Clinically Available Testing

The non-diagnostic clinical testing capabilities today consist of genetic, serological and inflammatory markers. The serological markers used are antibody specific and can be useful in aiding in the diagnosis of patients with histologically classified IC (Danese and Fiocchi 2011; Conrad et al. 2014). Table 9. below summarizes the laboratories and tests offering IBD associated testing.

Table 9. Current Available IBD Testing

Lab	Test	Methodology	Detection Rate	Gene List	Indications
Emory Genetics	Inflammatory Bowel Disease: Sequencing Panel	Next Generation Sequencing	~99%	ADAM17, AICDA, ATG16L1, BTK, C1orf106, CD40LG, COL7A1, CYBA, CYBB, DCLRE1C, DOCK8, ERAP2, FERMT1, FOXP3, FUT2, G6PC3, GUCY2C, HPS1,HPS4, HPS6, ICOS, IL10, IL10RA, IL18RAP, IL23R, IL2RA, IRGM, ITGAM, LRBA, MEFV, MVK, NCF2, NCF4, NOD2, PIK3R1, PLCG2, PTEN, PTPN22, RAC2,RB1, RET, SH2D1A, SLC37A4, STAT1, STXBP2, TTC37, WAS, XIAP	Very early onset IBD or IBD-like intestinal inflammation.
Quest Diagnostics	Inflammatory Bowel Disease Differentiation Panel	Immunoassay	pANCA+/ASCA– 50% sensitive and 35% specific for UC pANCA–/ASCA+ 47% sensitive and 31% specific for CD	ANCA screen with reflex to ANCA Titer, Myeloperoxidase Ab (MPO), Proteinase-3 Antibody, Saccharomyces cerevisiae Ab (ASCA) IgG, Saccharomyces cerevisiae Ab (ASCA) IgA	Intended to aid in the diagnosis of Crohn's Disease. A positive pANCA aids in the differentiation of patients with ulcerative colitis
Prometheus Therapeutics and Diagnostics	IBD sgi Diagnostic	Combines serologic, genetic and inflammation markers	Sensitivity: IBD 74% CD 89% UC 98% Specificity: IBD 90% CD 81% UC 84%	**Serological Markers**: anti-Fla-X, anti-A4-Fla2, anti-CBir1, anti-OMPC, and DNAse-sensitive pANCA **Genes:** ATG16L1, STAT3, NKX2-3, ECM1 I**nflammation Markers**: VEGF, ICAM, VCAM, CRP, SAA	Aids healthcare providers in differentiating IBD vs non-IBD and CD vs UC
Prometheus Therapeutics and Diagnostics	Crohn's Prognostic	Serologic and Genetic markers	Not provided	**Serologic Markers:** anti-CBir1, anti-OMPC, and DNAse-sensitive pANCA **Genes:** NOD2 variants SNPs 8,12,13	Quantifies a patients individual probability of developing disease complications over time
Center for Genetics at Saint Francis	Crohn's Disease - NOD2/CARD15 Complete Gene Analysis	Bi-directional Sanger Sequence Analysis	99%	**Gene:** NOD2/CARD15	Identify any gene variants
Prometheus Therapeutics and Diagnostics Baylor Medical Genetics Laboratory				**Gene**: TPMT (thiopurine methyltransferase) **Genotypes**: High/High = normal enzyme activity High/Low = intermediate activity Low/Low = low to no activity	A qualitative evaluation, to determine

Table 9. Continued

Molecular Genetics Laboratory Cincinnati Children's Hospital Laboratory Boston Children's Hospital	TPMT	Genotype or Enzymatic Analysis	99%	**Enzyme**: thiopurine S-methyltransferase **Reference Range:** Normal Activity: >21.0 EU/mL - Intermediate Activity: 6.0 - 21.0 EU/mL - Low Activity: <6.0 EU/mL	a patient's ability to utilize thiopurine drug therapy

The information in the above table displays the available IBD testing in the US. Data for this table was collected from the following websites: NCBI GTR: Genetic Testing Registry, Quest Diagnostics, Emory Laboratory, and Prometheus Therapeutics and Diagnostics.

4.6.3.1 Serological Markers

High levels of neutrophil activity are common in cases of UC and Crohn's colitis although is not typical in classic CD cases. Perinuclear anti-neutrophil antibody (pANCA) or atypical ANCA (xANCA) reactivity is found in 50 – 70% of UC patients compared to 10% of CD cases (Conrad et al 2014). A yeast antibody called Anti-Saccharomyces cerevisiae (ASCA, glycan antibody) is more reactive in CD vs. UC cases (Beaugerie and Sokol 2012). Anti-goblet cell antibody (GAB) is another UC specific marker occurring in 15–28% of UC patients (Conrad et al. 2014).

Three additional markers are used to identify antibodies to specific bacteria. " . . . Escherichia coli outer-membrane porin C (OmpC), Pseudomonas fluorescens CD-related protein (anti-12) and the CBir1 flagellin (CBir1)." (Beaugerie and Sokol 2012). Specific combinations of these markers can provide information regarding not only disease type but also behavior, i.e. reactivity to the three bacteria antibodies plus ASCA is associated with not just CD but early disease onset and need for small bowel surgery (Beaugerie and Sokol 2012).

4.6.3.2 Laboratory findings

Laboratory features (elevated ESG, CRP and faecal calprotectin) are not disease specific markers but rather indicators for general inflammation and deficiencies due to malnutrition (iron deficiency, anaemia) but can be helpful in assessing disease activity (Conrad et al. 2014).

4.6.4 Validity of Current Testing

Genetic testing is very useful in Mendelian genetic disorders, such as cystic fibrosis, in which changes in a single known gene lead to disease symptoms. Genetic test results in these cases provide a molecular diagnosis, sometimes even in an asymptomatic individual. However, a diagnosis of IBD cannot be made from a genetic test alone; it still relies on the incorporation of clinical signs and symptoms. This limitation makes a diagnosis of an asymptomatic individual through genetic testing impossible. Furthermore, current genetic testing for IBD remains invalidated minimizing its clinical utility even in symptomatic individuals.

Gerich and McGovern (2014) explain that the general population prevalence of IBD is about 0.6%, which correlates into a low pre-test probability for the average person. In an individual with IBD, the utility of testing is small, as the available methods are not considered diagnostic and colonoscopy and endoscopy are still needed to make a diagnosis. They also presents an interesting example pertaining to familial IBD stating that not all IBD associated SNPs correlate directly to disease in any given individual. They demonstrate this in a hypothetical but plausible scenario of a mother and daughter with CD, the daughter has a NOD2 disease-associated variant and the mother does not, suggesting that the daughter's CD is obviously not caused by a NOD2 mutation and in that case possibly neither is the mother's. In this case the daughter's 'risk score' would not have been a true reflection of her genetic risk (Gerich and McGovern 2014). In

conclusion, they states until high power studies can validate predictive phenotype – genotype findings it is premature to offer any kind of direct to consumer testing.

Danese and Fiocchi (2011) state that genetic screening for UC would be more useful if the there were fewer implicated genes and those genes had greater effect sizes. Lissner and Siegmund (2013) discuss the need for better risk stratification in order to separate high risk from low risk individuals and that current biomarkers cannot provide a valid stratification model.

4.6.5 Cancer Risks

The primary cancer risk associated with IBD is colorectal cancer (CRC). Individuals with a diagnosis of UC are more likely to develop CRC than individuals with CD. Margo et al. (2013) states that the incidence of CRC is approximately 4/1000 per person year of disease and the average prevalence is 3.5%.

Other factors that increase CRC risk are disease location and severity. Individuals with pancolitis or Crohn's colitis are more likely to develop CRC. A younger age at diagnosis, presence of PSC, a family history of CRC (independent of IBD), and the presence of pseudopolyps all increase the CRC risk. Duration of disease is a crucial factor with risk increasing at a rate of .5 to 1% per year starting 8 to 10 years after diagnosis (Cummings and Rubin 2006; Margo et al. 2013). Cummings and Rubin (2006) suggest that the risk may be modified by undergoing cancer surveillance colonoscopy with biopsies every one to three years.

Individuals with CD can be at risk for the rare case of small bowel carcinoma, with lesions typically occurring in the distal jejunum and ileum (Margo et al. 2013; Cummings and Rubin

2006). Margo et al. (2013) explains that due to the rarity of developing this type of cancer surveillance is not recommended.

4.6.6 Psychosocial Considerations

The majority of IBD cases result in a mild disease course, allowing many individuals to lead a normal life with minimal disturbances and ability to reach their social and professional life goals (Ordas et al. 2012; Cummings and Rubin 2006).

For more severely affected patients medical intervention is critical for obtaining a normal life; however, because of the heterogeneity of IBD, treatments are not always effective and disease symptoms can weigh heavily on all aspects of a person's life. Simple things such as traveling outside the home, social activities with friends and especially eating out may be difficult or nearly impossible, which may lead to feelings of depression and anxiety. The potential inability to work, attend school or take care of one's family can also become great stressors in the life of an individual with severe IBD. It is important as a genetic counselor to be aware of these issues and provide appropriate support and resources for these patients.

There are many national support services and resources available to IBD patients. The most prominent resource in the IBD community is the Crohn's and Colitis Federation of America (CCFA). Founded in 1967, this non-profit organization is a large financial contributor to IBD research and provides the latest educational and support services for patients and families. The CCFA offers 40 support groups nationwide that provide those with IBD the opportunity to meet and share information. There is a connecting database specifically designed for college students to meet other college students with IBD. A mobile diary app called "GI Buddy" was created to help patients keep track of symptoms and medications.

Over 200 Facebook pages and groups are dedicated to connecting those with IBD, Crohn's and/or UC. Individuals also reach out through YouTube, an online video sharing website to in an attempt to connect and share information. This type of patient-to-patient communication can be very helpful to reduce the feelings of isolation, anxiety and depression that may be accompanied by IBD. Patients also utilize these portals to discuss the benefits and side effects of different medications, or diet trends like the Specific Carbohydrate Diet and juicing recipes. In whatever way a patient chooses to access support, it is important to remind him or her to utilize these portals with caution and to always verify any medical information they discover with their doctor.

Another consideration to note is that conversations about bowel movements may be embarrassing, and it is not uncommon for individuals (especially children) to hide or ignore their symptoms. Lack of medical attention is especially serious in young people, as the disease has been shown to progress more rapidly and behave more aggressively in childhood, making parental awareness key. As a genetic counselor, a family history positive for IBD warrants a discussion of the risks and early signs and symptoms (loss of appetite, abdominal pain, weight loss, more frequent trips to the bathroom and recurrent constipation). The majority of early-onset cases do have positive family histories, and thus a parent personally affected by IBD may be more aware than a non-affected parent. It is always a good idea to discuss with parents what to look for and how to open the lines of communication with their children.

5.0 FUTURE STUDIES

As research delves further into the underpinnings of IBD, we are learning that the pathogenesis of this disease is strikingly complex. In order to determine the underlying pathology, we need to gain a greater understanding of the cell types involved in the initiation and progression of disease, how those cells are regulated and utilized in human tissue, and how they lead to specific phenotypes (Knights et al. 2013).

Due to the number of identified risk loci and the large number of involved pathways, it has been suggested that the current method for categorizing individuals (based on clinical manifestations) may not be sufficient. As we begin to unravel the complexities of IBD genetics and immune system pathways, we may find that individuals are better categorized by the specific pathway that is dysregulated. Soon there may be several subtypes of UC, several subtypes of CD, and previous cases of IC once thought to be "indeterminate" may become a third major category of classification (Hanauer 2006). Patients classified by pathway involvement may invite a personalized medicine approach, which touts better and more specific disease treatment that ideally targets the underlying cause and not merely the symptoms.

In addition, further research still needs to assess genetic and serological testing in unaffected cohorts of diverse ethnic backgrounds as preliminary research shows lack of congruency between European cohorts and other ethnic groups. There is a great need for all future studies to include rigorous phenotype data, especially if classification by pathways begins to create new disease subtypes. Knowing the associated phenotypic features will be necessary for clinical application (Benitez et al. 2014).

Deeper investigation into the microbiome is greatly needed to answer the question of what triggers the loss of tolerance to commensal bacteria. The answer may lie in the metagenome and further study into how it and the host genome interact. Longitudinal studies cataloguing microbiome composition before and after disease will aid in that regard (Jostins et al. 2012; Graham and Xavier 2013; Knights et al. 2013).

A current frustration in genetic research of IBD is that suspected loci do not consistently coincide with disease manifestations. It remains unclear if this is an issue of incomplete penetrance, polygenic inheritance, or some combination of the two. It has been useful to examine genetic variants in both affected and unaffected cohorts to tease out loci that may be appropriate for clinical genetic testing. In the future reliable diagnostic testing will improve treatment efficacy or affected individuals but also offer options for surveillance and family planning to both affected and unaffected persons.

6.0 CONCLUSION

In conclusion, there are still many unanswered questions about IBD, which can be frustrating for both physicians and patients. The disease has an increasing incidence worldwide, simultaneously increasing the number of individuals requiring medical services, time, support and education. This creates a need for medical professionals to accommodate this rapidly expanding patient population, and genetic counselors may be an appropriate option.

The skills of genetic counselors are broad and highly adaptable such that they would be a valuable contribution to any IBD clinic or research team. They are the best individuals to navigate the genetics of IBD, which are much more complex than the more straightforward Mendelian diseases. GI physicians, like many other specialists, are under pressure to keep up with constantly evolving genetic landscape as well as detection and treatment modalities. This presents an additional time commitment that may be a burden to already overwhelmed GI physicians. A genetic counselor is trained with a capacity to synthesize pertinent research and translate it into understandable terms suitable for patients. This ability to break down complex information can be an asset to research coordination as well, assisting research teams in connecting to and educating a motivated patient population.

While current genetic testing options have some clinical utility for affected patients they are not yet appropriate for widespread clinical use in unaffected individuals. The majority of genetic research on causative genes has been done on Caucasian populations of European descent and replication studies have provided inconsistent findings IBD populations of minority ethnic groups. The failure to identify universal candidate genes reduces the utility of the tests in the

minority ethnic groups making the role of a genetic counselor extremely useful in situations where genetic testing is being offered to such patients.

As both the technology and knowledge surrounding IBD genetics continues to expand along with the IBD community's awareness of the benefits and capabilities of genetic testing the role of genetic counseling will become more practical and applicable to the GI specialty.

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