A review of Crohn's Disease Gene-Environmental Interaction

"A study of gene-environment interaction between genetic susceptibility variants in

NOD2 and cigarette smoking in Crohn's disease etiology"

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Crohn's Disease Gene-Environmental Interaction

Statement of the Problem

Crohn's disease is a chronic inflammation in a genetically susceptible individual of any part of the gastrointestinal tract (mouth to anus) due to inappropriate inflammatory response or loss of immune tolerance to intestinal commensal microbes (Hedin et al, 2011). The condition was described in 1932 by Crohn, Ginzber, and Oppenheimer as terminal ileitis since the disease most commonly affects the distal small intestine. The disease second most common site is the proximal large bowel (Abraham & Cho, 2009). Crohn's disease (CD) is a form of inflammatory bowel disease characteristically presenting diarrhea, abdominal pain, and weight loss with numerous complications including abscesses, strictures, fistulas, and extra- intestinal involvement (Ahmed et al, 2011). The etiology of CD is poorly understood and its pathogenesis is complex consisting of a combination of factors which include intestinal inflammation from abnormal immune reaction to bacterial intestinal flora resulting in tissue injury, genetic susceptibility factors, and undetermined environmental factors which puts the susceptible individual at a higher risk (Shanahan, 2003). Certain infectious agents may act as cofactors may trigger the disease initiating the mucosal immune response such as childhood measles and *Mycobacterium pseudotuberculosis* (Shanahan, 2003). However, recent studies on the implications of *Mycobacterium pseudotuberculosis* causing ileitis have given inconclusive results   (Abraham & Cho, 2009). Increased permeability of the intestine may play a role in disease activity, genetic predisposition, and response to therapy (Inca et al, 2006).

Increase in both incidence and prevalence of the disease have been observed in the last 50 years with an increased frequency in the more developed countries such as Western Europe and North America and with increasing numbers observed also in less developed countries with

progressive industrialization. The apparent incidence increase has been seen as infectious

diseases decrease and the development of diagnostic techniques such as endoscopic

examinations increase (Logan, 2014). It has been hypothesized that the rise in prevalence of CD

may be due to elements in the changing environment, sanitation improvements, little of no

consumption of non-fermented foods, sterile foods, first exposure to intestinal pathogens, decline

in infections with helminthes, and vaccination might affect the proper development of the

mucosal immune system (Shanahan, 2003). The prevalence of CD in industrialized societies is in

excess of 0.1 percent with higher incidence in northern latitudes affecting the young up to 25

percent being first diagnosed before their 18th birthday (Hedin et al, 2011). Recent incidence of

the disease has increased substantially in children 10 to 14 year-old from 30 to 50 per million

mostly due as the result of developments in technology allowing early diagnosis rather than an

increase in this age group (Logan, 2014). The median age of diagnosis is 30 years old with a

bimodal age of distribution of 15 to 30 year- old and 60 to 70 year-old. The female to male ration

of CD is 1.2:1 being more common in Caucasians (Abraham & Cho, 2009). There is a high

prevalence in Ashkenazi Jews with family history of CD in about 15 to 20 percent of patients

(Shanahan, 2003).

The association of an increase in mortality of CD diagnosis in individuals at an early age

as well as when later in life remains controversial. Population based studies have found that

standardized mortality ratio (SMR) defined as "the ratio of observed deaths to age and sex-

matched expected deaths from the same catchment population" was increased in CD diagnosed

as teenagers (Selinger & Leong, 2012). Causes of death in Crohn's disease extracted from death

certificates include smoking related causes such as lung cancer and chronic obstructive

pulmonary disease, reflecting that smoking is a CD risk factor which may be a confounding

factor (Selinger & Leong, 2012). About a third of deaths in CD are attributed to non-malignant gastrointestinal disorders related to CD or its treatment such as pancreatic, gallstone related disease, and genitourinary tract disorders (Selinger & Leong, 2012).

According to Hedin et al (2011) CD is a polygenic disease, therefore; the risk of developing the disease significantly increases by having a parent or sibling with CD. The relative risk of developing CD for a sibling of a patient with the disease is 35 times background population risk. The risk increases to up to 36% likeness of getting the disease where both parents are have CD. In twins CD heritability is high with a risk up to 50 percent in monozygotic twins. Performing family studies of CD patients allows for environmental and behavior comparisons between patients and unaffected persons of the effects of birth order, breastfeeding, childhood environment, smoking, and diet to be elucidated. According to Shanahan (2003) the best documented environmental influence in CD phenotype and susceptibility is smoking. Studying these biomarkers on siblings has the advantage over studying CD patients because the confounding effects of disease are removed allowing the exploration and investigation into the pathogenesis of disease onset, prediction, and disease prevention.

Molecular Basis of Crohn's Disease

Multiple genes are involved in CD susceptibility to the disease as well as to its distribution and clinical course which explain the complexity of the disease. Genome-wide association studies have found approximately 71 genetic loci indicating predisposition to CD (Hedin et al, 2011). The discovery of the *CARD15* gene, also termed NOD2 nucleotide-bonding oligomerization domain containing 2 (HuGeNavigator, 2014) illustrated the interaction between genes, the immune system and intestinal bacteria. The *CARD15/NOD2* gene is located on chromosome 16 (*IBD* locus) and consists of caspase activation recruitment domains (CARD), a

central nucleotide-binding domain and a leucine rich domain which acts as a receptor for lipopolysaccharide and peptidoglycan found in bacteria. There are three coding regions variants associated with CD: Leu1007fsinsC/3020insC, Arg702Trp/C2104T, and Gly908Arg/G2722C. These three polymorphisms simplified as R702W, G908R, and 1007fs lie within or near a leucine rich domain. *CARD15* is associated with CD at a younger age and acts in an autosomal recessive fashion. In a case-control study conducted by Inca et al (2006) to examine genetic predisposition and the role of intestinal permeability in Crohn's disease, the authors found higher rates in *CARD15* mutations in familial versus sporadic healthy relatives associated with abnormal permeability in CD. Inca et al (2006) also found genetic variants in the *CARD15/NOD2* gene strongly associated with susceptibility to CD. According to Hruz & Eckmannb (2010)

Findings

Helbig et al (2012) in the article titled "A case-only study of gene-environment interaction between genetic susceptibility variants in NOD2 and cigarette smoking in Crohn's disease aetiology" the authors used a case- control study design to investigate in patients with CD the potential gene-environmental interaction between cigarette smoking and susceptibility variants in *NOD2*. The authors hypothesized that the interaction between disease-associated *NOD2* alleles and history of smoking would deviate from multiplicativity with regard to risk increase for CD thus being potentially indicative of biological interaction and that the deviation would be more pronounced among patients who were active smokers at the time of CD diagnosis. Individuals with Crohn's disease (CD) recruited through physician referrals based on a positive diagnosis established by clinical, radiologic, and endoscopic criteria to confirm the diagnosis of CD which constituted the case definition. The authors excluded criteria were non-

proband members of multiplex families and thus related to other members of the study sample.

Of the multiplex families, only the index patients were included in the final study sample.

Participants were included in the analysis only if genotype data were available for all three CD-

associated NOD2 polymorphisms. Participants were identified across Germany from the period

1995 to 2010 for a total of 2430 of which 1636 or 67 percent were eligible for participation. 514

were men or 31.4 percent and 1122 were women consisting of 68.6 percent. Mean age at

diagnosis was 26 year-old and mean age at inclusion was 39. Ethnicity of the participants was

not described. Using a control population from the popgen biobank as a comparison group, the

authors confirmed smoking as a risk factor for Crohn's disease in the study population (CI 1.24

(1.04-1.47) $p = 0.016$ for ever having smoked. The number of participants with the smoking

environmental exposure data consisted of total participants 1636: ever smokers 945 (57.8%),

never smokers 691 (42.2%), smokers at diagnosis 522 (40.75), non-smokers at diagnosis 761

(59.3%). A peripheral venous blood sample was obtained from each participant at time of

recruitment with informed consent from which DNA was extracted by TaqMan® SNP

genotyping for R702W, G908R, and 1007fs gene polymorphisms.

Demographic and risk factor variables for the included and excluded participants were compared

using X^2 test for categorical variables t-test for continuous variables. The authors employed a

case-control study design not a single case design to estimate statistical gene-environment

interactions under a multiplicative model. Logistic regression analysis was performed to assess

departure from multiplicativity between the presence of NOD2 polymorphisms and smoking

status. Odds ratios were used to indicate departure from multiplicativity between risk allele under

consideration and smoking status for the interaction term in the regression model and respective

95% confidence intervals (CIs). Conservative Bonferroni correction at an overall alpha level of

0.05 was applied to all analyses to correct for multiple comparisons. The gene-environmental interactions from the analysis showed a significant negative interaction between carriership of at least one of the NOD2 risk alleles and history of ever having smoked (OR = 0.71; p = 0.005) as well as smoking at the time of CD diagnosis (OR = 0.68; p = 0.005). Subsequent separate analyses of the three variants revealed a significant negative interaction between the 1007fs variant and history of ever having smoked (OR = 0.64; p = 9 × 10-4) and smoking at the time of CD diagnosis (OR = 0.53; p = 7 × 10-5).

Helbig et al (2012) concluded that the gene-environmental interaction of cigarette smoking and CD was significantly negative indicating that interaction of cigarette smoking and the NOD2 polymorphisms as risk for developing CD is smaller than expected indicating a possible biological interaction. The authors indicated the need for further epidemiological and functional studies to elucidate pathophysiology of the disease and for the more accurate development of disease prevention recommendations.

Discussion

The results obtained in Helbig et al (2012) study

Conclusions

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