

Polymorphism of IL10RA (S159G) Associated with Late-Onset Ulcerative Colitis

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ABSTRACT Inflammatory bowel disease (IBD) is a group of disorders distinguished as Crohn's Disease (CD) and Ulcerative colitis (UC). The etiology is multi-factorial and numerous genes are involved in the pathogenesis of IBD; hence the genetic cause is only moderately understood. Interleukin-10, a candidate gene of UC is activated by binding of IL10RA and IL10RB. The objective of the study is to examine IL10RA polymorphism in two patients with late onset of UC with an increased risk for development of Colorectal Cancer (CRC). Case 1 is a 35-year old male presented with UC for past 6 months who underwent colonoscopy which showed inflammatory ulcers, inflammation and pseudopolyps. Case 2 is a 37-year old female undergoing UC treatment for past 6-7 years and the colonoscopy revealed pseudopolyps in colon and rectum. Genetic analysis was performed by PCR-RFLP which revealed polymorphism in IL10RA with an SNP S159G (A/G) in exon 4. When compared with previous reports, the present data deciphers that in Indian population IL10RA is a novel mutated gene in UC.

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are the inflammatory bowel disease (IBD) which causes gut inflammation. CD can affect the entire part of the digestive system while UC targets the colon (large intestine). IBD is widespread in western countries affecting 1 out of 250 people (Martina et al. 2014). From the 20th century, the incidence and prevalence of IBD have increased (Molodecky et al. 2012). UC is characterized by anomalous inflammation of the inner surface of the rectum and colon, which causes ulcers in the large intestine. The cause

of UC is unknown, but previous studies stated that it could be due to one possible cause which is the immune system malfunction. The early onset of colitis is seen in 18-30 years and the late-onset after the age of 30. Hart (2010) stated that early onset appears to have a genetic component whereas late-onset is due to the changes in the immune system and environmental factors. The most common symptoms of UC are abdominal pain, cramping and frequent diarrhea with blood, pus, or mucus in the stools. Chronic hemorrhage from the inflamed and ulcerated intestinal tissue can cause anemia in some affected individuals. People with this disorder have difficulty in absorbing fluids and nutrients from the diet and often experience weight loss.

Genome wide associated studies (GWAS) in murine models explicate the genetic complexity underlying the IBD (Atsushi and Emiko 2008). The studies on genetics of UC are less understood, since there are a number of genes involved. The recurrent genes implicated are interleukin 10 receptor subunit alpha (IL10RA) and interleukin 10 receptor subunit beta (IL10RB). IL-10 is an anti-inflammatory cytokine secreted by an array of cell types and it is important for maintaining immune homeostasis in the gas-

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traintestinal tract (GIT). IL-10 activates by binding to two receptor subunits (Martina et al. 2014), comprised of two α subunits (IL10RA) and two β subunits (IL10RB) (Christopher et al. 2013). The two subunits activate JAK1 and TYK2 which lead to phosphorylation, nuclear translocation of STAT3 and gene transcription (Christopher et al. 2013). The protein coded by IL10RA is specific to IL10 receptor whereas IL10RB codes a subunit of receptors for several cytokines (Sidney et al. 2004). IL10RA, related to interferon receptors, possibly reconcile the immunosuppressive signal of interleukin 10, and inhibits the synthesis of proinflammatory cytokines. Concurrently IL10RB is an accessory chain essential for the activity of interleukin 10 receptor complex. Co-expression of IL10RA and IL10RB proteins has been shown to be required for IL10-induced signal transduction.

Objective

The present study explores the genetic make-up involved in ulcerative colitis by performing molecular analysis to examine the polymorphism, using PCR-RFLP technique. Generally, the diagnosis of ulcerative colitis is complicated because its symptoms may emulate other intestinal disorders. A previous report shows that IL10RA involvement is seen only in early onset UC, whereas the current report explains its association in late onset patients also.

CASE REPORTS

Ulcerative colitis is an atypical clinical disease and the following two cases were referred to the researchers department for genetic studies. The researchers obtained the questionnaire and signed consent from the patients before proceeding to genetic analysis. Pedigree analysis was performed and both the cases did not reveal any family history of UC.

Case 1

A 35-year-old male was suffering from abdominal pain and diarrhea for past 6 months. He had history of abdominal pain and distension, loss of appetite, tenesmus, weight loss and passing loose stools more than six times/day. He did not reveal any clinical symptoms of fever, dysphagia and dyspepsia. There was blood and

mucus discharge in stools. Laboratory examinations were conducted, in which blood investigations showed haemoglobin of 9.8g/dL, total blood count was 5800 cell/w.mm with neutrophil of 66.2 percent and lymphocyte of 27.8 percent, blood glucose of 144mg/Dl, blood urea of 20mg/dL and total protein of 4.6gm/dL. Colonoscopy showed pseudopolyps with inflamed mucosa (Fig. 1). Colonoscopic biopsy confirmed inflammatory polyps. Upper gastrointestinal endoscopy tests showed gastritis. His medications included mesacol 1.2gm/day and prednisone 5mg/day.



Fig. 1. Inflamed colonic mucosa with multiple pseudopolyps

Case 2

This case is a 37-year-old female who is undergoing treatment for UC since 2006. The patient was suffering from abdominal pain, mucus and blood in stool, tenesmus and low grade fe-



Fig. 2. Loss of mucosal vascular pattern with ulcerations and pseudopolyps

ver and no history of dysphagia, indigestion and loss of weight. Blood investigations revealed hemoglobin of 10.3gm, WBC of 8900cells/w.mm, blood glucose of 144mg/dL, blood urea of 14mg/dL and total protein of 6.4gm/dL. Colonoscopy results showed inflamed mucosa with loss of vascular pattern and pseudopolyps of varying sizes in the transverse colon, splenic flexure, descending colon, sigmoid colon and rectum (Fig. 2). Colonoscopic mucosal biopsy test showed the active phase of ulcerative colitis. Endoscopy results were normal. Currently, she is being treated with mesacol 400mg and prednisone 5mg.

From the clinical reports, the blood samples were collected and paraffin embedded tissue blocks studied for molecular analysis. DNA was isolated by Lahiri et al. (1993) method for blood medium and Larissa et al. (2011) for tissue and the isolated DNA was genotyped using PCR-RFLP technique. IL10RA genotyping was performed and allele-specific PCRs were used to detect the SNP. The polymorphism was observed with the substitution of serine (S) to glycine (S159G) with the nucleotide change of A/G at exon 4 region of IL10RA.

DISCUSSION

The major trigger that commenced the era of molecular genetics in IBD is based on epidemiological findings and the observed concordance within twin pairs (Weronica et al. 2014). In two of the researchers case studies, they analyzed a variant in IL10RA gene which is strongly associated with IBD. The variant S159G caught the attention in causing UC. Loss of function of IL10, IL10RA and IL10RB in immune-deficient patients is associated with severe and infantile-onset of IBD (Christopher et al. 2013). This has improved the interest in learning IL-10 pathway genes in the pathogenesis of IBD. Several experimental studies evidenced that IL10R signaling pathway protects the intestine from inflammation. Due to the mutation in the receptors, the IL10 immunomodulatory signal is cancelled, which leads to intestinal hyperinflammation (Christopher et al. 2013). IL10RA is expressed in many cell types of innate and adaptive immune system. Further studies are needed to analyze the type of cells involved in intestinal immunity (Erik-Oliver et al. 2009). Till date, much data is not available on the association between the variants and early on-

set of UC. Here, for the first time, the researchers are reporting about the role of IL10RA in late onset of UC.

Crohn's disease and UC are considered to be related disorders where they occasionally share same genetic loci but differs at times (Richard et al. 2006). The gene NOD2 (nucleotide-binding oligomerization domain 2) commonly causes CD and has the potential to play a role in UC too. Polymorphism in NOD2 was seen with 38.2 percent in CD and 13.7 percent in UC (Annese et al. 2005). In Indian population the studies on NOD2 revealed that NOD2 was weakly associated with UC with four SNPs (Srinivasan et al. 2012). Conversely, this case study has remarkably revealed the polymorphic changes in IL10RA. Recent report stated a novel variant in NOD2 gene and two missense variants in IL10RA (S159G and G351R) to be a causative factor for early onset of IBD. But this study was not contented and planned, to draft large number of patients for better understanding of the genotype-phenotype correlation (Martina et al. 2014). This study has similarity to the researchers' analysis, in which the variant S159G was also seen. One of the subunits of interleukin 10 receptor IL23R is found to be associated with CD on chromosome 1p31 and also with UC (Richard et al. 2006). The association of IL10RA with two variants S159G and R351G has shown loss of function (Grundtner et al. 2009), which is similar to the researchers case. In a mice model, the S159G variant in IL10RA showed a loss of function allele for IL-10-induced STAT1 and STAT3 activation but did not protect from susceptibility to UC (Grundtner et al. 2009).

An imperative goal of genetic evaluation for IBD is the development of individualized defensive strategies based on the genetic risk assessment, with the patient's personal medical history, and lifestyle preferences. In particular, the etiology of UC is a multifaceted which includes environmental and genetic causes. All of these factors should be taken into account in the risk assessment process. Mental health counseling and support groups can be extremely helpful in dealing with the psychological impact of IBD. Clinically, patients with UC and a family history of IBD are more likely to be diagnosed at the younger age. Currently, there are no conventional assessments (such as UC and CD associated antibodies) for possible identification of relatives at greater risk for developing IBD. The

identification of the polymorphism association with IL10R (S159G (A/G)) has provoked meaningful progress towards understanding the genetics of UC. As the IL10R polymorphism has been reported in a number of case reports, in future, genetic counseling, target directed therapeutic modalities, and also preventive strategies will be possible.

CONCLUSION

In conclusion, typically IL10RA is described in early onset UC, but in the present case study, it can be speculated that the mutation of IL10RA is seen in late onset which is responsible for the decreased activity of the receptor. Consequently, from previous studies the variation determines a dysfunctional effect in causing ulcerative colitis. Ulcerative colitis is commonly seen in younger people and the diagnosis is often made in people between the ages of 15 and 30. Less frequently it is seen in people who are aged above 30. These two novel cases presented with clear phenotypic evidence of ulcerative colitis and emphasize the environmental risk factors in determining the disease phenotype. However, the researchers are unable to identify the exact external factors involved in the expression of UC. The presence of polyposis may be a risk factor for CRC, hence careful follow-up study is recommended in both the cases. Thus, to the best of their knowledge, these case reports emphasize the association of IL10R polymorphism (S159G (A/G)) in ulcerative colitis.

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