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Research Article

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An Analysis and Survey on Interleukin-10 Receptor Mutation in Inflammatory Bowel Disease in Iranian IBD Cohort

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Abstract

Background: *Early-onset inflammatory bowel disease (IBD) is classified to Crohn's disease, ulcerative colitis and unclassified disorders which have chronic, relapsing course and can result in substantial long-term morbidity. IBD is a multifactorial disorder with genetic susceptibility, immunological predisposition and environmental triggers.*

Objective: *To generally determine prevalence of IL10R mutation in IBD patients in Iran-Isfahan, we performed sequencing of all exons in IL10RA and IL10RB in cohort of IBD patients and healthy control.*

Material and Method: *Total DNA content of 76 patients and 50 healthy controls were extracted from whole blood and PCR amplification and sequencing was done.*

Result: *Overall identified IL-10RA mutations were P.(I224V), P.(A153V), P.(A153A), P.(S159G), P.(R263Q), P.(R284C), P.(R351Q), P.(Q376Q), P.(T416I), P.(A493V), P.(A511A) and P.(S563S).*
In IL10RB gene the only detected mutation was P. (K47E).
Of them, P.(A153V), P.(A153A), P.(R284C), P.(T416I), P.(A493V), P.(A511A), P.(S563S) were Not reported variant in IBD variants.

Conclusion: *Our results also confirmed that early-onset IBD could be attributed to a synergistic effect of several variant alleles of the genes encoding IL10 receptors. These variants, alone, could only give rise to a sub-clinical manifestation of the IBD.*

Keywords: Inflammatory bowel disease, Immunodeficiency, Single nucleotide polymorphism, Interleukin 10 receptor, Monogenic, Mutation

Introduction

Inflammatory bowel disease (IBD) is classified to Crohn's disease (CD), ulcerative colitis (UC) and unclassified which has a chronic, relapsing period and may result in substantial long-term morbidity [1]. IBD is a multifactorial disorder with genetic susceptibility, immunological predisposition and environmental triggers [2]. To date, more than 210 genetic loci have been associated to IBD [3,4]. Genetic loci implicated in IBD shows several pathways that are crucial for intestinal homeostasis, barrier function, epithelial restitution, microbial defense, innate immune regulation, reactive oxygen species (ROS) generation, autophagy, regulation of adaptive immunity, endoplasmic reticulum (ER) stress and metabolic pathways associated with cellular homeostasis [2,5].

In IBD, monogenic causes need special consideration. To identify high risk patients, specific surveillance (e.g. for malignancy or infection), or specific treatments (e.g. bone marrow transplant) is required [1]. Monogenic conditions causative variants in interleukin-10 (IL10), IL-10 receptor (IL10 R), X-linked inhibitor of apoptosis protein (XIAP), ADAM metallopeptidase domain 17 (ADAM17), Neutrophil Cytosolic Factor 4 (NCF4) and Tetratricopeptide Repeat Domain 7A (TTC7A) were identified in very early onset IBD (VEOIBD) patients, the data presenting the hypothesis that severe infantile colitis frequently starting immediately after birth might represent a group of heterogeneous monogenetic diseases [6]. Among these candidate genes, IL-10 and IL-10R gene mutations have been extensively investigated [7]. IL-10 is an anti-inflammatory cytokine secreted by a variety of cell types and is necessary for maintaining immune homeostasis in the gastrointestinal (GI) tract.

IL10 has role in restricting T cell proliferation, down-regulating co-stimulatory protein expression on antigen-presenting cells, and limiting pro-inflammatory cytokine production. IL-10 activates downstream signaling by binding to the IL-10 receptor (IL-10R), comprised of two α subunits (encoded by IL10RA) and two β subunits (encoded by IL10RB). This activates Janus kinase 1 (JAK1) and tyrosine kinase 2 (Tyk2), leading to phosphorylation and nuclear translocation of signal transducer and activator of transcription 3 (STAT3) and gene transcription [8].

It is well known that a substantial geographical and ethnical variation in IBD incidence exist worldwide. The incidence of IBD is steadily rising in many regions of the world however in Iran the incidence and prevalence rates of IBD are not available. So, we established the first IBD bio bank in Iran and the process of IBD patient's registration is ongoing [9].

Single nucleotide polymorphisms (SNPs) that affect IL-10 production have been associated with CD and/or UC. As far as we are concern, there is no data available for evaluation of IL10R mutation in IBD patient in Iran. We focused on patient's candidate gene study by sequencing of IL10RA and IL10RB in IBD patients.

Material and Methods

1. Patient Information

Patients were referred to the immunodeficiency research center and Al-Zahra university hospital at the Isfahan University of Medical Sciences.

Diagnosis of IBD was based on standard tools including clinical features, endoscopy with biopsies and adequate imaging of the small bowel. According to the Paris classification we collected their clinical characteristics, including disease type, sex and age of onset and disease behavior. Information pertaining to family history, medical treatment, surgical intervention including the age at operation and history of infections was also obtained. Furthermore, history of immunodeficiency and autoimmunity in the patients considered. Blood samples from 76 patients and 50 healthy donor controls were collected upon informed consent.

2. DNA Extraction and PCR Amplification

Total DNA content of each patient was extracted from whole blood with the Siam DNA Blood Mini Kit (Qiagene#51104). PCR amplification was done as previously described [10]. Initial denaturation was done at 95°C for 5min, and then 30 cycles denaturation at 95°C for 30s, annealing at 59°C for 30s and elongation at 72°C for 1 min, after cycles final elongation at 72°C for 10 min.

The PCR reactions were performed in a 20ml volume containing 100ng genomic DNA and 2.5 picomoles of each primer. 5ul of each reaction was sent for sequencing. Primers set used for PCR reaction were prepared from Kotlarz, et al. paper (Table 1) [2].

Table 1: Primers set used for PCR

Name of primer	5' → 3'
IL10RA 1-F	GACAGTGGTTCCCCGTCC
IL10RA 1-R	CACTGGATGGAGAACTTTAATGG
IL10RA 2-F	GAACCTCCCTTTCTTCTTTGG
IL10RA 2-R	AGGCAGGTATCTTCCCATGC
IL10RA 3-F	GGCCTCTTGCGTCTCCC
IL10RA 3-R	GCAGACATGGTGAGCTATGG
IL10RA 4-F	TCCGTGGACTAATTGTTCTGC
IL10RA 4-R	AGTCCATAAGGTGCTGCCAC
IL10RA 5-F	AAGTCTAAAACGGCTATTATCACTG
IL10RA 4-F	TCCGTGGACTAATTGTTCTGC
IL10RA 4-R	AGTCCATAAGGTGCTGCCAC
IL10RA5-F	AAGTCTAAAACGGCTATTATCACTG
IL10RA 5-R	AGCTGGAATTTGAGTTGGATG
IL10RB 1-F	AGGGTAAAGAAGACCCTCAAA
IL10RB 1-R	CCTAGTTGCGTCTCAGCAG
IL10RB 2-F	GGAGAACCAAGTGCTGGATG
IL10RB 2-R	CAGACTCCCTTCCTCCTGTG
IL10RB 3-F	TTAACACAGTTTCCACTCCCG
IL10RB 3-R	AAGGCCATCCATTTGTGG
IL10RB 4-F	TCCGTGGACTAATTGTTCTGC
IL10RB 4-R	AGTCCATAAGGTGCTGCCAC
IL10RB 5-F	AAGTCTAAAACGGCTATTATCACTG
IL10RB 5-R	AGCTGGAATTTGAGTTGGATG
IL10RB 6-F	GGCTCTGTTTTCAGGGATTG

IL10RB 6-R	CATGTTGTCTGGAATTGGGC
IL10RB 7-1F	TCCAGCCAGGAGTTCTGTG
IL10RB 7-1R	GCTGAAAATTACACTCTCAGTGG
IL10RB 7_2F	CTCCCAGACCCTGGACTTAG
IL10RB 7-2R	TCACTTTGTCACCCAGGC
IL10RB 7-3F	GATGGCGCATGCCTATAATC
IL10RB 7-3R	TGGACATCAAGATGGCAAAC

Results

To determine prevalence of IL10R mutation in IBD patients in Iran-Isfahan, we performed sequencing of all exons in IL10RA and IL10RB in cohort of IBD patients and healthy control (Table 2).

Table 2: Main clinical characteristics of IBD patients and HCs

	CD	UC	HC
Number	16	60	50
Female	10(62.5)	26(43.3)	26
Male	6(37.5)	34(56.7)	24
Age mean (SD)	22(12.5)	21(15.1)	19(16.4)
Age at diagnosis mean (SD)	16.6(10.3)	13(6.5)	-
Corticosteroid-dependent – n (%)	12(75)	44(73.3)	-
Corticosteroid-refractory – n (%)	4(25)	17(26.7)	-
Autoimmunity– n (%)	4(25)	22(36.6)	-
Immunodeficiency– n (%)	2(12.5)	10(16.6)	-
Consanguinity– n (%)	10(62.5)	24(40.0)	17(34)

All consisting of single base pair substitutions were identified and confirmed by Sanger sequencing (Table 3).

Table 3: Frequency of SNPs in IBD patients

SNP	Chromosome position	Alleles	Ancestral Allele	Prevalence	MAF	Function
SNP in exon IL10RB						
rs2834167	117994131	A/A	A	3	0.5	Missense (K47E)
SNPs in IL10RA						
rs2228055	117994131		G	1	0.1	Missense (I 224 Val(
rs752413943	117993331	C/T	Not reported	2	Not reported	Missense (Ala 153Val)
rs2256111	117993332	A/G	A	25	0.4832	Synonymous (Ala153Ala)
rs3135932	117993348	A/G	A	11	0.0817	Missense (Ser159Gly)
rs145975996	117995688	A/G	G	5	Not reported	Missense (Arg263Gln)
rs755029073	117998754	C/T	Not reported	1	Not reported	Missense (Arg284Cys)
rs2229113	117998955	A/G	G	23	0.1889	Missense (Arg351Gly)
rs747711495	117999032	A/G	Not reported	3	Not reported	Synonymous (Gln376Gln)
rs200254237	117999151	C/T	C	4	0.0002	Missense (Thr416Ile)
rs746081732	117999382	C/T	Not reported	1	Not reported	Missense (Ala493Val)
rs751283486	117999437	C/T	Not reported	1	Not reported	Synonymous (Ala511Ala)
rs768512760	117999593	A/G	Not reported	1	Not reported	Synonymous (Ser563Ser)

Overall detection rate of IL-10RA mutations was 69.3% (53/76) and IL10-RB 3.9 (3/76) in total patients. Identified IL-10RA mutations were P. (I224V), P. (A153V), P. (A153A), P. (S159G), P. (R263Q), P. (R284C), P. (R351Q), P. (Q376Q), P. (T416I), P. (A493V), P. (A511A) and P. (S563S). IL10RB mutation was P. (K47E). Of them, P. (A153V), P. (A153A), P. (R284C), P. (T416I), P. (A493V), P. (A511A), P. (S563S) were Not reported variant in IBD variants.

The most common mutations were p. (A153A) and p. (R361G) which found 63.1% (48/76) patients.

Discussion

The immune response has evolved to protect the host from a broad range of pathogenic microorganisms and control over control immune responses and prevent reactivity to self are required to limit host damage to keep hemostasis [11].

Intestinal homeostasis is a highly dynamic process requiring sensitivity to mount appropriate immune responses toward microbial or food antigens, yet necessitating the regulation of these responses in order to prevent chronic inflammation.

Cytokines have an important role in the pathogenesis of IBD; they control multiple aspects of the inflammatory response. In particular, the imbalance between pro-inflammatory and anti-inflammatory cytokines in IBD could cause of the resolution in inflammation and tissue destruction [12].

IL-10 as an anti-inflammatory cytokine plays role in keeping gut homeostasis secreted by various cells, including monocytes, macrophages, T and B lymphocytes, dendritic cells, epithelial cells, and mast cells [11]. IL10 mediates its anti-inflammatory effects through IL-10R-dependent signals emanating from the cell surface.

The IL-10R is a hetero-tetramer that consists of two subunits of IL-10Rα and two subunits of IL-10Rβ. IL-10Rα subunit is unique for IL-10 signaling but the IL-10Rβ subunit is shared by IL-22, IL-26 and IFN-λ receptor [13]. After binding IL-10 to its receptor, it activates JAK1 and Tyk2, leading to the phosphorylation of STAT-3, the activation of downstream target genes, and finally the expression of anti-inflammatory effectors [14].

In study of Shim, et al. incidence of p. (T84I) reported as a trigger to IBD pathogenesis [11]. Moran, et al. confirmed association of p. (I224V) variant with IBD. Study of Glocker et al indicated that IBD may be a monogenic disorder and homozygous mutations of p. (W159X) in IL-10RB were found in Kurdish siblings with consanguineous parents [15]. In our study we could confirm p. (T84I), p. (I224V) and p. (W159X) variant in our patients. Genetic alteration which caused by p. (R263Q) variant can affect IL10 receptor intracellular signaling and is related to IBD pathogenesis [13]. In five patients P. (R263Q) variant was detected in our study. P. (R351Q) genetically predisposed IBD incidence that we also reported in our study [14]. To the best of our knowledge P. (A153V), P. (A153A), P. (R284C), P. (T416I), P. (A493V), P. (A511A), P. (S563S) variants were Not reported in IBD variants.

Single nucleotide polymorphisms (SNPs) that affect IL-10 production; have been associated with CD and/or UC. For the first time our results confirmed that early-onset IBD could be attributed to a synergistic effect of several variant alleles of the genes encoding IL10 receptors in Iranian IBD patient. These variants, alone, could only give rise to a sub-clinical manifestation of the IBD.

Suggestion

In Iran, as a result of high rate of consanguinity, incidence of genetic based disorders is relatively high; therefore evaluation of IL10R mutation in IBD patient is necessary. This recommendation may help patients to have precisely diagnosis and tailed therapy.

Consent

DNA Sanger sequencing of genes encoding for IL-10R1, IL-10R2, and IL-10 was performed upon written informed consent.

RKH, FV and SN designed and performed experiments, evaluated and interpreted data, M.N statistical analyses, ME, HS, HT and PA contributed patients, MY, NN, MB, collected clinical data and samples, or screened cohorts RSH, AR, DK and CK gave conceptual advice.

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