RESEARCH ARTICLE

Novel Mutations in IL-10 Promoter Region -377 (C>T), -150 (C>A) and their Association with Psoriasis in the Saudi Population

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Abstract

Background: Psoriasis, a common cutaneous disorder characterized by inflammation and abnormal epidermal proliferation with a prevalence of 2-3% in the general population, may be linked to certain types of cancer. Several studies have reported an association between interleukin 10 (IL-10) variant polymorphisms and inflammatory diseases such as psoriasis vulgaris although the results vary according to the population studied. No studies have been performed in the Saudi population. The present study concerned novel variants and other genetic polymorphisms of the promoter and exonic regions of the IL10 gene in patients with moderate to severe psoriasis and potential differences in genotype compared to a group of healthy volunteers. Materials and Methods: Patients with moderate to severe psoriasis and healthy controls with no personal or family history of psoriasis were selected from the central region of Saudi Arabia. Polymorphisms of the IL 10 gene of both groups were genotyped. Results: We observed two novel variants in 5'UTR region of the promoter precursor with higher prevalence of the genotype with both wild-type alleles in patients compared to the healthy control group. The differences at positions -377 and -150 were significantly associated with disease, both the variants conferred strong protection against psoriasis in Saudi patients. Conclusions: This observation provides further support for the importance of the part that IL10 plays in the pathophysiology of this disease. Confirmation of our findings in larger populations of different ethnicities would provide evidence for the role of IL-10 in psoriasis.

Keywords: Psoriasis - IL-10 - promoter region - genetic polymorphisms - Saudi population

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Introduction

Psoriasis is a chronic inflammatory skin disease characterized by infiltration of inflammatory elements, keratinocyte hyperproliferation, and altered differentiation, which may be directly or indirectly linked to cancer development, for example in the cervix (Kim et al., 2014) and skin (Pouplard et al., 2013), as well as non-Hodgkin lymphoma (Pouplard et al., 2013). The etiology of psoriasis remains unclear, various factors including genetic susceptibility and environmental effects have been involved (Nestle et al., 2009; Wang et al., 2013). Psoriasis is affecting 2-3% of the population worldwide (Langan et al., 2012). Approximately 25% of patients also develop psoriatic arthritis, a common, debilitating auto-immune disease belonging to the family of spondyloarthritides (Liu et al. 2008). The pathogenesis of psoriasis and psoriatic arthritis is complex, involving both genetic and environmental risk factors. Strong association of psoriasis with the MHC class I region and DNA repair genes were demonstrated in the 1990s and has been confirmed in numerous subsequent studies (Bethea et al., 1999). Genetic variation in the multiple histocompatibility locus antigen cluster (MHC) and DNA repair genes increases risk of developing psoriasis. However, only ~10% of individuals with MHC risk factor develop psoriasis, indicating that other genetic effects and environmental triggers are important. Defects in the interleukin 10 (IL-10) signaling pathway have been shown to cause very early onset psoriasis.

Interleukin-10 (IL10) gene is located on chromosome 1 at 1q31-1q32 region, codes for anti-inflammatory cytokine. Interleukin-10 is encoded by five exons, covering 4.8 kb transcribed to form a 1.6 kb mRNA encoding a 178-amino-acid protein. IL10 cytokine is primarily produced by monocytes and to a lesser extent lymphocytes; namely type2 T helper cells (Th2), mastocytes, CD4+CD25+Foxp3+ regulatory T cells, and a certain subset of activated T cells and B cells (Moore et al., 2001; Dennis, 2013). IL10 has a stimulatory effect on certain T cells (Th2), mast cells and it stimulates the B cell survival, proliferation and antibody production (Moore et al., 2001; Sabat et al., 2010). The IL10 play a role in different diseases, such as; cancer, cutaneous

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malignant melanoma, skin squamous cell carcinoma and inflammatory bowel diseases (Tagore et al., 1999; Howell et al., 2001; Hanappa et al., 2002; Alamartine et al., 2003; Abhimanyu et al., 2011; Acuner-Ozbabacan et al., 2013; Neurath, 2014). The mutational status of IL-10 gene in Saudi Arabian psoriasis patients is still obscure. On the basis of these considerations, we designed this study to investigate whether the variants IL-10 could be risk factors for the development of psoriasis in Saudi patients, hence we sequenced all the five exons and promoter region.

Materials and Methods

Subjects

This was a prospective study in patients with moderate to severe plaque psoriasis, defined as a psoriasis area and severity index greater than or equal to 10 and/or an affected body surface area greater than 10%, with or without psoriatic arthritis. All patients were resident in Riyadh province, central region of Saudi Arabia .This study included clinically diagnosed plaque psoriasis patients recruited during the period from March 2012 to December 2012 from patients admitted to the dermatology department, King Khalid University Hospital, Saudi Arabia. All subjects underwent complete history taking. All patients were not receiving systemic treatment or biologic therapy for psoriasis for at least six months before analysis. Healthy Saudi volunteers served as a control group. Control individuals were healthy and unrelated to one another, with no first- or second-degree relative with psoriasis.

DNA extraction

Approximately 3 ml of blood samples were collected in sterile tubes containing ethylenediaminetetracetic acid (EDTA) from all subjects enrolled in the study. Genomic DNA was isolated from blood samples using QIAmp kit (QIAmp DNA blood Mini Kit, Qiagen, Valencia, CA) following the manufacturer's instructions. After extraction and purification, the DNA was quantitated on a NanoDrop 8000, to determine the concentration and its purity was examined using standard A260/A280 and A260/A230 ratios (NanoDrop 8000) (Sambrook et al., 1989).

IL10 sequencing

Reference genomic sequence was retrieved from the Ensembl database [www.ensembl.org]. Primers for PCR and sequencing of five exons and promoter region were designed using Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast) and synthesized commercially (Macrogen, Korea) (Table 1). We have amplified the target regions using primer pairs (Table 1) and an amplitaq gold PCR master mix (Life technologies). The reactions were carried out in an ABI GeneAmp PCR system 9700. The thermal cycling parameters used were as follows: initial denaturation at 95ºC for 5 minutes, followed by 35 cycles of denaturation at 94ºC for 1 minute, annealing for 30 seconds and elongation at 72ºC for 1 minute (Table 2). PCR amplification was followed by Exo-SAP treatment (Affymetrix, USA), following manufacturer's protocol. Exo-SAP treated amplicons were sequenced directly using BigDye terminator (v.3.1) cycle sequencing kit (Applied Biosystems, USA) on an ABI 3730XL DNA analyser. Sequence variations were identified by assembling DNA sequences with the reference sequence using AutoAssembler software (Applied Biosystems, USA). Variations obtained were validated and reconfirmed in a subset of samples by re-sequencing and visual confirmation of electropherograms.

Statistical analysis

Genotype and allelic frequencies were computed and were checked for deviation from Hardy-Weinberg

Table 1. Primer Sequence and Annealing Temperature of IL10 Primers

Gene	Primer	Tm
IL10 PF	5'-ATCCAAGACAACACTACTAA-3'	48°C
IL10 PR	5'-TAAATATCCTCAAAGTTCC-3'	
IL10 E1F	5'-AGAGGCCTCCCTGAGCTTAC-3'	49°C
IL10 E1R	5'-GTTTGGGGGAATAGGTGTTG-3'	
IL10 E2F	5'-GCATCAAAAAGACCGCATTT-3'	50°C
IL10 E2R	5'-CCCTTAATCATGCTGCACAC-3'	
IL10 E3F	5'-AGCAGCCAGAGGGTTTACAA-3'	50°C
IL10 E3R	5'-TGCTGTGTCTGTGGATGTGA-3'	
IL10 E4F	5'-CACCAGCTTGTCCCCTAAGT-3'	50°C
IL10 E4R	5'-GCAGCGAGCAGTCATTTAGA-3'	
IL10 E5AF	5'-CTCTGCACTCAAGGTCATGC-3'	50°C
IL10 E5AR	5'-AGGTCAGGGAAAACAGCTCA-3'	
IL10 E5BF	5'-CTGTTTCCATAGGGTGACACA-3'	50°C
IL10 E5BR	5'-TGGTTGGGGAATGAGGTTAG-3'	
IL10 E5CF	5'-TTGGGGCTTCCTAACTGCTA-3'	50°C
IL10 E5CR	5'-GCAGAATTCATTCACCCACTC-3'	

Table 2. Genotype Distribution of IL10 Gene Polymorphism in Psoriasis Cases and Controls

Genotype		Cases (%)	Controls (%)	OR	95%CI	X^2	p- value
IL10 -377 C>T	CC	67	25	Ref			
	CT	20	75	0.1	0.016-0.627	6.74	0.00943
	TT	13	0	1.667	0.063-43.78	0.58	0.44752
	CT+ TT	33	75	0.167	0.031-0.904	4.64	0.03131
	C	77	63	Ref			
	T	23	37	0.507	0.155-1.655	1.28	0.25727
IL10 -150 bp C>A	CC	60	25	Ref			
	CA	27	75	0.148	0.026-0.860	4.89	0.027
	AA	13	0	1.842	0.07-48.68	0.64	0.42503
	CA+ AA	40	75	0.222	0.042-1.175	3.31	0.06896
	C	73	63	Ref			
	A	27	37	0.606	0.191-1.927	0.73	0.39437

equilibrium (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). Case-control and other genetic comparisons were performed using the chi-square test and allelic odds ratios (OR), and 95% confidence intervals (CI) were calculated by Fisher's exact test (two-tailed). Statistical analysis was done using SPSS 16.0 for Windows. We considered p-value of <0.05 as significant (Alanazi et al., 2013).

Results

Patients diagnosed with psoriasis based on clinical and laboratory findings were included in the present study. All the patients were examined by a dermatologist and informed consent was obtained from patients for genetic studies. Their age ranged between 18-60 years. Their mean age and standard deviation (SD) was 37.50±8.08 years. The PASI score for clinical assessment ranged between 9 and 63, the mean±SD was 33.2±18.3. No mutations were detected in the IL-10 gene in the controls. In our search to understand the association of IL10 with psoriasis we sequenced both IL10 promoter and exonic regions, but we did not find any mutations in the coding region of the IL10 gene in Saudi psoriasis patients.

We have observed two novel variants in Saudi psoriasis patient IL-10 promoter region at -377 bp (Figure 1) and -150 bp upstream to the 5' UTR region when compared with reference sequence (ref|NC_018912.2|) and control samples. To determine the frequency of these IL10 polymorphisms, we sequenced these regions in more number of psoriasis and healthy Saudi population. The genotype and allele frequencies of the investigated IL-10- promoter polymorphisms in patients and controls are summarized in table 2. The minor allele frequencies and genotype frequencies are shown in table 2. Genotype frequencies were following Hardy-Weinberg equilibrium in cases.

The polymorphisms of IL-10 exhibited very strong association with psoriasis patients (p <0.05) when genotypes were compared using chi squared test 3 x 2 contingency table 2. Both -377 bp and -150 bp were associated with reduced risk of psoriasis. Compared to the homozygous wild genotype 'CC' in the IL-10 -377 bp, the heterozygotes 'CT' showed decreased risk of developing psoriasis (OR, 0.10; CI, 0.016-0.627; p=0.00943). The overall comparison with CT+TT allele in the IL-10 at -377bp in promoter region also provided modest protection against psoriasis (OR, 0.167; CI, 0.031-0.904; p=0.03131). The heterozygous CA allele in the IL-10 at -150 in 5' UTR region also showed strongest protective association with Saudi psoriasis patients when compared to homozygous CC allele (OR, 0.148; CI, 0.026-0.860; p=0.027).

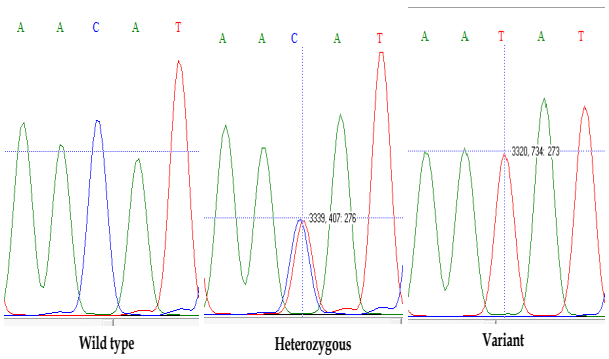

Figure 1. -377 C>T mutations (a) wild type C allele (b) heterozygous CT allele (c) Variant T allele

Discussion

Psoriasis is a genetically heterogeneous disorder with multiple genetic and environmental interactions. Based on its genetic framework, disease severity and locations may differ between individuals and populations (Burden et al., 1998, Karam et al., 2014). As cytokines are important immune mediators, we tested SNPs in the promoter regions of two cytokine genes for association with this chronic inflammatory skin disease. IL-10 is an anti-inflammatory cytokine that suppresses macrophage production of cytokines/chemokines and enhances soluble cytokine receptor release adding that IL-10 modulates antigen presentation by dendritic cells and suppresses co-stimulatory reactions by a direct action on T cells (Jacob et al., 2003).

In this study, we have analyzed IL-10 promoter and exonic regions to identify novel mutations associated with psoriasis patents in Saudi population. To the best of our knowledge this is the first study to report about IL-10 -377(C>T), -150 (C>A) novel variants with predisposition to psoriasis in Saudi Arabian population. IL-10 -377(C>T), -150 (C>A) are novel mutations that have not been previously reported to be associated with Psoriasis. We have observed that IL-10 -377(C>T), -150 (C>A) variants are highly significant reduced risk of developing psoriasis. Our finding of the strong protection conferred by the -377(C>T), -150 (C>A) variants of IL-10 promoter region against psoriasis though in a small population size is significant and merits examination of this SNP in larger studies of different ethnic groups.

The present study has certain limitations and few strengths. The most important is the small sample size both for patients and controls. In addition, the fact that we only assessed those patients with moderate to severe psoriasis and that we did not adjust for whether psoriatic arthritis was present. However, the population size in our study is small, the findings are significant that need to be confirmed in larger and ethnically different groups for the identified potential markers to be used for psoriasis cancer screening. A similar study with a larger patient cohort, stratified based on the age and severity of the disease might provide the influence of genetic variants in the inflammatory genes of Saudi Arabia is needed to determine whether these results can be extrapolated to the Saudi population as a whole.

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