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Association of genetic polymorphism of interleukin-17A & interleukin-17F with susceptibility of psoriasis

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***Background & objectives*: Psoriasis is a chronic inflammatory skin disease with unknown aetiology. So far studies have confirmed that interleukins, pro-inflammatory factors and T-cell activation play major role in the development of disease. Interleukin-17 (IL-17) a T helper inflammatory cytokine, was found to be positively correlated with severity of psoriasis. However, the specific mechanism has not been clarified. IL-17A and IL-17F are group members of IL17 family cytokines and found to be located adjacent to one another on the same human chromosome, 6p12. The present study was designed to identify the association between *IL-17A* and *IL-17F* gene polymorphism with susceptibility of psoriasis in north Indian population.**

***Methods*: A total of 166 psoriasis patients and 150 healthy controls were genotyped for *IL-17A* and *IL-17F* gene polymorphism by amplification refractory mutation system-polymerase chain reaction method. One single nucleotide polymorphism (SNP) was analysed in IL-17A (rs10484879) and one SNP in IL-17F (rs763780) to look for an association with psoriasis.**

***Results*: Our study indicated decreased frequency of *IL-17A* (rs10484879) G allele (51.8 vs. 65.0%), and *IL-17F* (rs763780) C allele (36.5 vs. 45.7%) in psoriatic patients as compared to healthy controls.**

***Interpretation & conclusions*: The present findings suggest that *IL-17A* (rs10484879) G/T and *IL-17F* (rs763780) C/T gene polymorphisms may contribute in pathogenesis of psoriasis. Further studies need to be done to confirm these findings.**

Key words Cytokine - gene polymorphism - interleukins - psoriasis - single nucleotide polymorphism

Psoriasis is an immune mediated inflammatory disease that affects the skin, joints, and nails of all age group of people[1]. It is a global health problem with at least 100 million individuals affected worldwide. The reported prevalence of psoriasis in different countries ranges between 0.09 and 11.43 per cent[2]. In India most of the prevalence studies of psoriasis are hospital based. It was reported that incidence of psoriasis among total skin patients ranged between 0.44 and 2.2 per cent, with overall incidence of 1.02 per cent[3].

Psoriasis was originally classified as a T helper 1 (Th-1) disease because of the cytokines involved in the Th-1 pathway[4]. In Th-1 pathway Th-1, Th-17 and Th-22 cell populations are expanded and stimulated to release inflammatory cytokines, including tumour necrosis factor-alpha (TNF-α), interleukin (IL)-17 and IL-22. All of these cell types are found to be present in psoriatic lesions[5]. These cytokines contribute in inflammatory mechanism of pathogenesis of psoriasis. IL-17A and IL-17F are members of IL-17 cytokine family comprising four other members, namely IL-17B, IL-17C, IL-17D and IL-17E, all these have varying homology[6]. IL-17A and IL-17F are produced by Th-17 cells in response to IL-23, neutrophils, mast cells and Tc17 cells. They are present on Human chromosome 6p12, adjacent to each other and known to act as homodimers or as IL-17A/F heterodimers[5,7].

IL-17 and other Th-17 cytokines are linked to the pathogenesis of diverse autoimmune and inflammatory diseases and play key role in coordinating local tissue inflammation[8]. The IL-17 receptor is expressed ubiquitously, and hence most cells can potentially respond to this cytokine[6]. In IL-17 and IL-23 pathways, IL-17 plays major role in pathogenesis of psoriasis. In psoriatic arthritis, IL-17A acts as key mediator of inflammatory pathway[9]. There are some other studies which report that both IL-17A and IL-17F regulate inflammatory response thus act as pathogenic agent for several immunoinflammatory diseases including psoriasis, psoriatic arthritis and rheumatoid arthritis[10,11].

Another major role of IL-17 has been identified as activation of inflammatory pathway *via* stimulating nuclear factor-κB (NF-κB). NF-κB is a major transcription factor that plays a crucial role in transcription of responsive inflammatory genes upon releasing[12]. It has been reported that IL-17, may stimulate the transcription factor NF-κB which in turn activates the inflammatory pathway[13]. Inhibition of IL-17 may act as blocking of such inflammatory pathway associated with psoriasis. In agreement with that some recent studies have reported that blocking IL-17 or its receptor results in improvement of psoriasis[9].

Genetic variations in inflammation-related genes, especially cytokines and their receptors may lead to an increase in expression of IL-17[14]. Some previous studies have reported that polymorphism of interleukin IL-1β, IL-6, IL-16 and IL-17A is correlated with pathogenesis of inflammatory diseases as well as psoriasis[15-17]. In this regard, it is reasonable to hypothesize that genetic variation in *IL-17A* and *IL-17F* may affect the expression or activity of these genes which in turn may influence the susceptibility and severity of psoriasis. Therefore, this study was undertaken to investigate the influence of polymorphism of genes of these two cytokines on the risk of psoriasis in north Indian population.

Material & Methods

This study was conducted in the department of Human Genetic, Punjabi University, Patiala, India, during January, 2014 to November, 2016. Clinically diagnosed patients of psoriasis, visiting skin outdoor patients (outpatient department) of Dermatology department of Government Rajindra Medical College & Hospital (GRMC & H), Patiala, Punjab, and Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India, were included in the study. A total of 42 patients and 67 healthy individuals visiting the OPDs, were selected from GRMC & H, Patiala, and 124 patients and 83 healthy individuals from PGIMER, Chandigarh. Sample size was calculated on the basis of genotype and allele frequencies which were obtained in the pilot study, done on 25 individuals in each arm taking 5 per cent level of significance at two tailed test, 80 per cent power of the study. The difference of proportion of these genotype frequencies varied for 0.12 to 0.16. The sample was calculated on minimum difference of 0.12, which provided 319 samples. A total of 250 psoriasis patients and 200 healthy volunteers were initially screened based on inclusion and exclusion criteria. Newly diagnosed psoriatic patient (prior to treatment) and those who had undergone off treatment for topical (for 2 wk), systemic and phototherapy (for 4 wk) were included in the study. Patients taking any treatment (allopathic, ayurvedic, homeopathic) for psoriasis, those suffering from any other coexistent autoimmune disorders, acute or chronic infections, and malignancies and those who were not willing to take part in the study, were excluded. Of the 250 patients, 166 of age 40.55±11.6 yr, having 109 (65.7%) males and 57 (35.3%) females and 150 healthy individuals of 38.78±6.8 yr as controls having 106 (70.7%) males and 44 (29.3%) females, were included in the study (Table I). All the patients and healthy individuals gave written informed consent and the study protocol was approved by the institutional clinical ethical committee of Punjabi University, Patiala.

Table I. Characteristics of psoriasis patients and controls

Characteristics	Mean (range or SD)	
	Patients (n=166) n (%)	Controls (n=150) n (%)
Age (yr)	40.55±11.6	38.78±6.8
Male	109 (65.7)	106 (70.7)
Female	57 (35.3)	44 (29.3)
Disease duration (yr)	8.92±8.2	-
BMI (kg/m^2)	23.97±5.1	21.80±3.48
PDI	21.02±6.5	-
PASI	16.21±11.9	-

BMI, body mass index; PDI, psoriasis disability index; PASI, psoriasis area severity index; SD, standard deviation

Isolation of genomic DNA and genotyping: Genomic DNA was isolated from the blood using phenol chloroform method[18]. A 3 ml of peripheral blood was drawn from each individual in sterile EDTA tubes for genomic DNA isolation. Genotyping for gene polymorphism of *IL-17A* and *IL-17F* was done using the amplification refractory mutation system (ARMS)-polymerase chain reaction (PCR) method[19]. PCR primers were designed by web-based allele specific primer designing tool based on primer3. In order to access the success of PCR an internal control, human growth hormone (HGH) was amplified using a pair of primers designed from the nucleotide sequence of HGH. The primer sequences used for amplification of *IL-17A* were as follows: allele-specific sense primer C 5'-GAT ATG CAC CTC TTA CTG CAC TC-3'; allele-specific sense primer T 5'- GAT ATG CAC CTC TTA CTG CAC TT-3'; antisense common primer 5'- AGTTGTACAGGCCCAGTGTA-3'; control primer (HGH) (sense), 5'- CCT TCC AAC CAT TCC CTT A-3'; control primer (HGH) (antisense), 5'- TCA CGG ATT TCT GTT GTG TTTC-3'.

Amplification was performed using a thermal cycler (Eppendorf, USA). PCR protocol for *IL-17A* was as follows ; 95°C for 1 min followed by 10 cycles of 95°C for 15 sec, 55°C for 50 sec, and 72°C for 40 sec, then 20 cycles of 95°C for 20 sec, 56°C for 50 sec and 72°C for 50 sec. PCR protocol for *IL17F* was as follows; 95°C for 1 min followed by 10 cycles of 95°C for 15 sec, 61°C for 50 sec, and 72°C for 40 sec, then 20 cycles of 95°C for 20 sec, 56°C for 50 sec and 72°C for 50 sec. The amplified products were separated by electrophoresis on a 2.0 per cent agarose gel stained with ethidium bromide. The gel was visualized under a ultra-violet transilluminator with a 100 base pair ladder and photographed.

Statistical analysis: Statistical analysis of the data was performed using Statistical Package for Social Sciences (SPSS, trial version 16.0) developed by IBM corporation on Java platform. The observed genotype distributions were compared with those expected from Hardy-Weinberg equilibrium using a standard Chi-squared test. Adjusted odds ratio (OR) for the genotypes was calculated after correction for psoriasis risk factors with binary logistic regression. All quantitative statistical analysis was carried out at 5 per cent value of significance.

Results

One hundred and sixty six psoriasis patients (male to female ratio 1.91:1) and 150 healthy controls (male to female ratio 2.1:1) were genotyped for *IL-17A* (rs10484879) and *IL-17F* (rs763780) gene polymorphism. Results showed that for *IL-17A* (rs10484879) gene polymorphism frequency of the G allele carriers was significantly decreased in psoriasis patients than that in healthy controls (51.8 vs. 65.0%), and for *IL-17F* (rs763780) gene frequency of the C allele carriers was found to be significantly decreased in psoriasis patients than that in healthy controls (36.5 vs. 45.7%), as shown in Table II. These results suggested that G allele might play a protective role against psoriasis for *IL-17A* (rs10484879) likewise C allele of *IL-17F* (rs763780) gene showed protective role against psoriasis.

Further analysis showed that individuals carrying the TT genotype and T allele of rs10484879 were more likely to have a significantly increased risk of psoriasis when compared with the GG genotype and G allele. The OR (95% confidence interval) for the TT genotype and T allele of rs10484879 were calculated as 2.7132 (1.465-5.027) and 1.7276 (0.882-1.524), respectively. However, no significant association was found between GT and GT+TT genotype of rs10484879 with psoriasis. Moreover, the TC and TT genotypes of rs763780 were associated with increased risk of psoriasis when compared with the CC genotype. The ORs (95% confidence interval) for the TC and TT genotypes were calculated as 2.4194 (1.2711-4.6052) and 2.5134 (1.2885-4.9027), respectively (Table II).

Discussion

Several screening studies and Genome-Wide association study (GWAS) have identified a large

Table II. Genotypes and allele frequencies of the interleukin -17A, and interleukin -17F gene polymorphisms in psoriasis patients and controls

Genotype	Control (n=150), n (%)	Cases (n=166), n (%)	*P*	Adjusted OR* (95% CI)
IL-17A (rs10484879)				
GG	67 (44.7)	55 (33.1)		Reference
GT	61 (40.7)	62 (37.4)	>0.005	1.2382 (0.749-2.046)
TT	22 (14.7)	49 (29.5)	<0.005	2.7132 (1.465-5.027)
GT + TT	83 (55.3)	111 (66.9)	>0.005	1.6291 (1.032-2.571)
G	195 (65.0)	172 (51.8)		
T	105 (35.0)	160 (48.2)	<0.005	1.7276 (0.882-1.524)
IL-17F (rs763780)				
CC	36 (24.0)	19 (11.5)		Reference
TC	65 (43.3)	83 (50.0)	<0.005	2.4194 (1.271-4.605)
TT	49 (32.7)	65 (39.2)	<0.005	2.5134 (1.289-4.903)
TC + TT	114 (76.0)	148 (89.2)	<0.005	2.4598 (1.341-4.514)
C	137 (45.7)	121 (36.5)		
T	161 (53.7)	213 (64.2)	>0.005	1.4979 (1.089-2.036)

*ORs were obtained from a binary logistic regression model. 95% CI, 95% confidence interval; ORs, Odds ratio

number of susceptibility genes involved in pathogenesis of complex disease like cancer, psoriasis, and other major human diseases[20,21]. Many studies have identified association of several susceptible genes including *IL-17* with psoriasis[22-24].

IL-17 is a pleiotropic pro-inflammatory cytokine which enhances T cell priming and stimulates epithelial, endothelial and fibroblastic cells to produce multiple pro-inflammatory mediators, including IL-1, IL-6, TNF-α and chemokines[25,26]. IL-17 has been found to be associated with the pathogenesis of a wide range of inflammatory and autoimmune diseases as psoriasis, rheumatoid arthritis, inflammatory bowel disease, systemic sclerosis as well as responses against bacterial and fungal infections[27].

During inflammation, both IL-17A and IL-17F are found to mediate pro-inflammatory responses[28]. Importantly, IL-17A must be considered within the context of the local microenvironment, because it acts synergistically or additively with other pro-inflammatory cytokines, including TNF[5]. Properties of IL-17 are largely dependent on the environment in which it is produced[25]. In agreement with some previous studies our results indicated that polymorphism of *IL-17A* (rs10484879) and *IL-17F* (rs763780) led to increased susceptibility of *IL-17* gene towards pathogenesis of psoriasis. The TT genotype and T allele of *IL-17A* rs10484879 and TC and TT genotypes of IL-17F rs763780 were associated with increased risk of psoriasis. According to our hypothesis increased susceptibility of psoriasis may be result of increased expression of IL-17 which causes the activation of NF-κB pathway. In addition, IL-17A upregulates expression of various other inflammation-related genes in target tissues (keratinocytes and fibroblasts)[5]. These keratinocytes and fibroblasts further increase production of various chemokines, cytokines, antimicrobial peptides and other mediators which mediate the pathogenesis of disease.

There were certain limitations of our study. The study was restricted to the north Indian region for sample collection from psoriasis patients, mainly from two hospitals of the region. PCR based method was used for detection of SNPs which may have errors that can determined by the sequencing.

In conclusion, our study showed that the *IL-17A* and *IL-17F* polymorphism was associated with psoriasis risk. Our study also suggested that polymorphism might contribute to disease by regulating the expression of IL-17. Although this was a small study, yet a strong association was observed between *IL-17A* and *IL-17F* gene polymorphism with psoriasis. It is, therefore, suggested that IL-17A might be an interesting target for development of new therapies for psoriasis in future.

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***Conflicts of Interest*:** None.

References

1. Griffiths CE, Barker JN. Pathogenesis and clinical features of psoriasis. *Lancet* 2007; *370* : 263-71.
2. World Health Organization. *Global report on psoriasis.* Geneva: WHO; 2016.
3. Dogra S, Yadav S. Psoriasis in India: Prevalence and pattern. *Indian J Dermatol Venereol Leprol* 2010; *76* : 595-601.
4. Moseley TA, Haudenschild DR, Rose L, Reddi AH. Interleukin-17 family and IL-17 receptors. *Cytokine Growth Factor Rev* 2003; *14* : 155-74.
5. Kirkham BW, Kavanaugh A, Reich K. Interleukin-17A: A unique pathway in immune-mediated diseases: Psoriasis, psoriatic arthritis and rheumatoid arthritis. *Immunology* 2014; *141* : 133-42.
6. Chang SH, Dong C. A novel heterodimeric cytokine consisting of IL-17 and IL-17F regulates inflammatory responses. *Cell Res* 2007; *17* : 435-40.
7. Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, *et al.* Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* 2006; *203* : 2271-9.
8. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, *et al.* Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006; *441* : 235-8.
9. Raychaudhuri SP. Role of IL-17 in psoriasis and psoriatic arthritis. *Clin Rev Allergy Immunol* 2013; *44* : 183-93.
10. Yao Z, Spriggs MK, Derry JM, Strockbine L, Park LS, VandenBos T, *et al.* Molecular characterization of the human interleukin (IL)-17 receptor. *Cytokine* 1997; *9* : 794-800.
11. AbuHilal M, Walsh S, Shear N. The role of IL-17 in the pathogenesis of psoriasis and update on IL-17 inhibitors for the treatment of plaque psoriasis. *J Cutan Med Surg* 2016; *20* : 509-16.
12. Verstrepen L, Carpentier I, Verhelst K, Beyaert R. ABINs: A20 binding inhibitors of NF-kappa B and apoptosis signaling. *Biochem Pharmacol* 2009; *78* : 105-14.
13. Gaffen SL. Structure and signalling in the IL-17 receptor family. *Nat Rev Immunol* 2009; *9* : 556-67.
14. Moorchung N, Vasudevan B, Chatterjee M, Mani NS, Grewal RS. Interleukin-1 gene polymorphisms and their relation with NFκB expression and histopathological features in psoriasis. *Indian J Dermatol* 2015; *60* : 432-8.
15. Prieto-Pérez R, Solano-López G, Cabaleiro T, Román M, Ochoa D, Talegón M, *et al.* The polymorphism rs763780 in the *IL-17F* gene is associated with response to biological drugs in patients with psoriasis. *Pharmacogenomics* 2015; *16* : 1723-31.
16. Patel DD, Lee DM, Kolbinger F, Antoni C. Effect of IL-17A blockade with secukinumab in autoimmune diseases. *Ann Rheum Dis* 2013; *72* (Suppl 2) : ii116-23.
17. Girolomoni G, Mrowietz U, Paul C. Psoriasis: Rationale for targeting interleukin-17. *Br J Dermatol* 2012; *167* : 717-24.
18. Di Pietro F, Ortenzi F, Tilio M, Concetti F, Napolioni V. Genomic DNA extraction from whole blood stored from 15- to 30-years at -20 °C by rapid phenol-chloroform protocol: a useful tool for genetic epidemiology studies. *Mol Cell Probes* 2011; *25* : 44-8.
19. Chen Q, Lu P, Jones AV, Cross NC, Silver RT, Wang YL. Amplification refractory mutation system, a highly sensitive and simple polymerase chain reaction assay, for the detection of JAK2 V617F mutation in chronic myeloproliferative disorders. *J Mol Diagn* 2007; *9* : 272-6.
20. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C. Complement factor H polymorphism in age-related macular degeneration. *Science* 2005; *308* : 385-9.
21. Bhandari P, Ahmad F, Mandava S, Das BR. Association of genetic variants in *ARID5B*, *IKZF1* and *CEBPE* with risk of childhood *de novo* B-lineage acute lymphoblastic leukemia in India. *Asian Pac J Cancer Prev* 2016; *17* : 3989-95.
22. Zhao Y, Forst CV, Sayegh CE, Wang IM, Yang X, Zhang B. Molecular and genetic inflammation networks in major human diseases. *Mol Biosyst* 2016; *12* : 2318-41.
23. Liu QP, Wu LS, Li FF, Liu S, Su J, Kuang YH, *et al.* The association between *GJB2* gene polymorphism and psoriasis: A verification study. *Arch Dermatol Res* 2012; *304* : 769-72.
24. Batalla A, Coto E, González-Lara L, González-Fernández D, Gómez J, Aranguren TF, *et al.* Association between single nucleotide polymorphisms *IL17RA* rs4819554 and *IL17E* rs79877597 and psoriasis in a Spanish cohort. *J Dermatol Sci* 2015; *80* : 111-5.
25. Martin JC, Baeten DL, Josien R. Emerging role of IL-17 and th17 cells in systemic lupus erythematosus. *Clin Immunol* 2014; *154* : 1-2.
26. Kolls JK, Lindén A. Interleukin-17 family members and inflammation. *Immunity* 2004; *21* : 467-76.
27. Shibata S, Saeki H, Tsunemi Y, Kato T, Nakamura K, Kakinuma T, *et al*. IL-17F single nucleotide polymorphism is not associated with psoriasis vulgaris or atopic dermatitis in the Japanese population. *J Dermatol Sci* 2009; *53* : 163-5.
28. Ishigame H, Kakuta S, Nagai T, Kadoki M, Nambu A, Komiyama Y, *et al.* Differential roles of interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses. *Immunity* 2009; *30* : 108-19.

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