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ORIGINAL ARTICLE

Association of *IL12B* risk haplotype and lack of interaction with *HLA-Cw6* among the psoriasis patients in India

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Psoriasis is a complex multifactorial chronic inflammatory skin disorder involving both genetic and environmental susceptibility factors. It is strongly associated with *HLA-Cw6*, but several studies suggested that further genetic factors may confer additional risk. We investigated the association of two single-nucleotide polymorphisms (SNPs), rs3212227 at the 3′-untranslated region and rs7709212 located at ~6.7 kb upstream from the transcription start site of *IL12B* gene in a case-control study comprising 1702 individuals from India. We found both SNPs were significantly associated with psoriasis (rs7709212: odds ratio (OR) = 1.37, *P*-value = 1.09×10^{-5}; rs3212227: OR = 1.38, *P*-value = 8.88×10^{-6}). *IL12B* gene was significantly upregulated in involved skin of psoriasis patients with risk genotype carriers (rs7709212_TT and rs3212227_TT) compared with non-risk genotype carriers (rs7709212_CC and rs3212227_GG). Significantly higher serum protein concentration of IL12 was also observed among risk allele carriers compared with non-risk allele carriers irrespective of the presence of *HLA-Cw6* allele. Haplotype analysis suggested significant increased risk (OR = 1.50, *P*-value = 5.01×10^{-8}) to the disease when both risk alleles of *IL12B* were present. IL12 serum protein concentration of risk haplotype (TT-TT) carriers showed significant upregulation compared with the non-risk carriers independent of *HLA-Cw6* alleles. Our data suggested the association of *IL12B* with the psoriasis, however no evidence was observed for the epistatic effect of *IL12B* with *HLA-Cw6* among the psoriasis patients in India.
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INTRODUCTION

Psoriasis is a chronic inflammatory skin disorder that affects individuals worldwide in all populations with a variable prevalence rate of 0–11.8%.[1,2] In India, the disease incidence is around 0.44–2.8%.[3,4] The exact cause of the disease is yet to be characterized, but it is considered to be a complex multifactorial disease involving both genetic and environmental susceptibility factors.[5] Previous studies suggested psoriasis to be an autoimmune disease mediated by T cells. Cross talk between the infiltrating immune cells and keratinocytes through inflammatory cytokines are suggested to cause the epidermal hyperplasia observed in psoriasis.[6] Genome-wide linkage scans and association studies identified involvement of several immune-related genes, highlighted the prominent role of genetics and immune system in psoriasis pathogenesis.[6–13] Genetic variations in different cytokines, their receptors and antagonists are shown to have major contribution in disease predisposition. Interleukin 12 (IL12), a pro-inflammatory cytokine produced by antigen presenting cells, is increased in psoriasis.[14] It regulates downstream adaptive immune response by promoting maturation of naive $CD4^+$ T cells toward Th1-phenotype.[15] IL12 is a heterodimeric cytokine, the biologically active p70 heterodimer is composed of p35 (encoded by *IL12A* gene) and p40 (*IL12B* gene) subunits.[16] IL12 is mainly secreted by activated monocytes/macrophages and dendritic cells, as well as by polymorphonuclear leukocytes and keratinocytes.[17] Simultaneous expression of both subunits in the same cell is required for its activity.[17,18] However, expression analysis showed that only p40 mRNA (from *IL12B* gene) levels were altered in psoriatic skin, whereas p35 was uniformly expressed in both normal and lesional skin of the psoriasis patients.[14]

A single-nucleotide polymorphism (SNP) (rs3212227) at the 3′-untranslated region (UTR) of *IL12B* gene was first reported from a small-scale Japanese study[19] and subsequently replicated in other populations.[20–23] However, contradictory observation was reported among the Mestizo population of western Mexico.[24] Expression pattern of *IL12B* was reported to alter with the risk genotype[18,25–27] or with the risk haplotype.[28] In contrary, other studies have shown IL12 (p70) protein concentration was not different in serum obtained from psoriatic patients and healthy controls among the Dutch, Mestizo and South Indian populations.[24,29,30] Another SNP (rs6887695) at the *IL12B* promoter was also not associated with psoriasis in Mestizo population.[24] Furthermore, IL12 p70 and p40 production did not vary upon lipopolysaccharide (LPS) treatment on blood cells derived from psoriatic and healthy individuals.[29] A recent study from South India among Tamil patients showed association with *IL12B* (rs3212227) and *IL23R* (rs2201841), but they did not observe

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any significant difference in IL12 protein concentration in the plasma of psoriasis patients having risk genotype compared with the non-risk genotypes carriers.[30]

HLA-Cw6 is the most strongly associated psoriasis susceptibility locus.[31–42] However, only 40–80% of affected individuals/families have shown association with this allele and penetrance of this allele is only ~10%.[5] This suggested that the combined effect of *HLA-Cw6* with other genetic or environmental factors may be involved in disease pathogenesis. Likewise, studies have also been conducted on possible epistatic effects of *IL12B* and *HLA-Cw6* among the psoriasis patients. Strong association of *IL12B* haplotype was reported, but no evidence of any statistically significant interaction between *IL12B* and *HLA-Cw6* was observed in the Caucasian population.[21] In contrary, another study on psoriasis patients from Chinese Han population showed a significantly increased (odds ratio (OR) = 36) disease risk among individuals carrying both *IL12B* and MHC risk alleles.[43] Several controversial reports of associations with *IL12B* genotype, epistatic effect with *HLA-Cw6* as well as differential expression pattern of IL12 suggested involvement of underlying population-specific effects of *IL12B* gene and its role in psoriasis pathogenesis. These variable reports prompted us to conduct the present study to determine association and expression pattern of *IL12B* on psoriasis patients from Eastern Indian cohort.

In this study, we investigated the association of two SNPs, rs7709212 located at the ~6.7 kb upstream from the transcription start site and rs3212227 in the 3'-UTR of the *IL12B* gene among the psoriasis patients in Eastern part of India. We also evaluated the interaction between *IL12B* and *HLA-Cw6* risk alleles among the psoriasis patients. Finally, we determined the functional effect of the risk alleles in terms of IL12 gene expression and serum protein concentration among the psoriasis patients with and without *IL12B* risk genotypes.

MATERIALS AND METHODS

Study population

Totally 1702 individuals with 814 psoriasis cases and 888 healthy controls from the Eastern region of India participated in the study. Mean age of psoriasis patients was 41 years (s.d. = 16.00) and the mean age of disease onset was 35.07 years (s.d. = 15.30), whereas control individuals had a mean age of 39.04 (s.d. = 15.06). Distribution of males in cases was 67.68% whereas in controls it was 51.29%. All patients were clinically diagnosed with psoriasis and confirmed by atleast two dermatologists. Only patients categorized as having plaque or guttate psoriasis were enrolled in the study to minimize clinical heterogeneity. Patients had mild to severe psoriasis. Healthy controls were recruited after they were clinically assessed as being without psoriasis, other autoimmune disorders, systemic disorders or without a family history of psoriasis in the first and second degree relatives. Written informed consent was obtained for all patients and controls; for children younger than 18 years of age, consent was also obtained from their parents. The study was approved by the Institutional Ethics Committee for Human Research, Indian Statistical Institute, Kolkata, India and conducted according to the Declaration of Helsinki Principles. Approximately, 3 ml of blood samples were collected from each individual. About 1.5 ml was transferred to clot activator-containing tubes for serum isolation, remaining half was kept in EDTA-containing vials for DNA extraction.

HLA-Cw6 allele and *IL12B* SNPs typing

Peripheral blood samples were collected and genomic DNA was extracted by Qiagen DNeasy Blood/Tissue DNA isolation kit according to the manufacturer's protocol. *HLA-Cw6* typing was carried out using sequence specific PCR (SSP-PCR) as described previously[42,44] and referred to as positive when the *HLA-Cw6* allele was present and negative when not present. We selected two previously reported non-coding SNPs near *IL12B* gene (located on 5q31-33): rs7709212,[20] 6696 bp upstream of *IL12B* gene and rs3212227 at 3′-UTR for possible association with disease. All SNPs were genotyped on a 7900HT Fast Real-Time PCR System Instrument by using allele-specific Taqman MGB probes labeled with fluorescent dyes FAM and VIC (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's protocols. Allelic discrimination was done with the ABI PRISM 7900HT SDS and the SDS 2.2.2 program (Applied Biosystems). Ten percent of the samples were run as duplicates to check for genotyping errors.

Haplotype analysis

Previous studies have shown that a larger genomic region (rs6887695–rs3212227), −60 kb 5′ to the 3′-UTR of *IL12B* gene was in linkage disequilibrium,[20,21] however, we limited our study to a smaller region (rs7709212–rs3212227), −6.7 kb 5′ to the 3′-UTR, which appeared to be more functionally relevant to our gene of interest. Haplotype analysis and linkage disequilibrium pattern was studied using the SHEsis online software platform (http://analysis.bio-x.cn).[45]

Gene expression analyses

Uninvolved and involved skin biopsies (4 mm) were obtained from 32 psoriasis patients with written consent. Eleven patients gave consent for the biopsy of both uninvolved and involved skin, and 21 patients only gave consent for biopsy of the lesional skin. Biopsy specimens were collected in RNA later (Invitrogen, Carlsbad, CA, USA) and stored at −80 °C until processing. For total RNA extraction, biopsies were snap frozen in liquid nitrogen and grinded to powder using mortar and pestle. RNA extraction was performed from 11 paired and 21 psoriatic skin tissue samples using AllPrep DNA/RNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Quality of the eluted RNA was checked in Nanodrop 2000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA). About 1 μg of total RNA was used for cDNA synthesis using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). The product was subsequently diluted and around 10 ng was finally used for each reaction. Transcripts were quantified using a 7900HT Fast Real-Time PCR system (Applied Biosystems) using Taqman probe-primers sets purchased from Applied Biosystems (*IL12B* Hs01011518_m1 and *GAPDH* Hs02758991_g1). All values were normalized to the expression of the housekeeping gene *glyceraldehyde 3-phosphate dehydrogenase (GAPDH)*.

Enzyme-linked immunosorbent assay

Serum was collected from 120 psoriasis patients and kept in −80 °C until further processing. Enzyme-linked immunosorbent assay (ELISA) was performed for IL12 in serum obtained from 52 patients with homozygous non-risk or risk allele carrier, using IL12A Single Analyte ELISA Kit (Qiagen) following manufacturer's protocol. Serum IL23 concentration was detected using Quantikine ELISA kit for Human IL23 (R&D Systems, Inc. Minneapolis, MN, USA) for 19 samples. The samples were replicated twice and the average of these replicates was considered for further analysis.

Statistical analysis

Case-control analysis was performed to test genetic markers for susceptibility to psoriasis. Hardy–Weinberg equilibrium was evaluated for each SNP using the χ^2-test. Association for genotype and allele frequencies between cases and controls were calculated using Pearson's χ^2-test. Fisher's exact probability test was used where expected count was less than five. Binary logistic regression analysis was carried out using R-programming. Interaction study was performed using multinomial logistic regression in SPSS software package (SPSS Inc., Chicago, IL, USA). Expression values were compared using unpaired *t*-test assuming unequal variance. Since the distribution of age and sex was different in cases and controls, we had also checked the association of SNPs after adjusting for age and sex using logistic regression analysis in R. Significant *P*-values were corrected for multiple testing using Benjamini–Hochberg multiple hypothesis testing correction in R (https://www.r-project.org/).

RESULTS

Association of SNPs near *IL12B* gene

We genotyped two non-coding SNPs, rs7709212 and rs3212227 near the *IL12B* gene among the 814 psoriasis cases and 888 healthy

individuals from the Eastern part of India. Approximately, 64% of the cases had early onset or type I (age of disease onset $\leqslant 40$ years), whereas 36% had late onset or type II (age of disease onset >40 years) form of the disease. These SNPs were individually found to be in Hardy–Weinberg equilibrium in both psoriasis cases and healthy controls (P-value >0.05). For both SNPs, the more frequent allele was observed to be the risk allele and was the same as that reported in earlier studies.[20–22] Furthermore, around 89% cases and 83% controls carried atleast one copy of the risk allele.

Significant association was observed for both SNPs at the genotype level (rs7709212: P-value $=9.41\times10^{-5}$; rs3212227: P-value $=5.11\times10^{-5}$) as well as at the allele level association analysis (rs7709212: P-value $=1.09\times10^{-5}$, OR $=1.37$; rs3212227: P-value $=8.88\times10^{-6}$, OR $=1.38$) (Table 1 and Supplementary Table 1). As age and sex distribution was found to be marginally different between cases and control, we determined the association of these SNPs after adjusting for age and sex. As shown in Table 1, we found similar observations before and after adjustment. In addition, patients with early and late onset forms (type I and type II) of the disease showed similar associations for both SNPs near the *IL12B* gene (Supplementary Table 2), unlike those observed in the UK cohort.[46] In an effort to elucidate the mode of inheritance, association pattern of both SNPs was estimated under dominant, recessive and additive models of association. Additive association model was found to be the optimal model for both SNPs (rs7709212: P-value $=9.41\times10^{-5}$; rs3212227: P-value $=5.11\times10^{-5}$), and were considered for all further genetic association analysis (Supplementary Table 3).

Linkage disequilibrium and haplotype analysis

As both SNPs had similar association patterns and are located around the same gene, we estimated the D' and r^2 values for rs7709212 located $\sim$6.7 kb upstream and rs3212227 located at the 3′-UTR of *IL12B* gene. Only a moderate level of linkage ($D'=0.702$ and $r^2=0.49$) was observed for these SNPs in Indian population, consistent with previous reports in North American population.[20] Haplotype analysis revealed that the most frequent haplotype with the risk alleles (rs7709212_T and rs3212227_T) was significantly higher in cases (P-value $=5.13\times10^{-8}$, OR $=1.50$ (95% confidence interval (CI): 1.30–1.74)), whereas the non-risk combination (rs7709212_C and rs3212227_G) was significantly less found in cases (P-value $=1.69\times10^{-4}$, OR $=0.74$ (95% CI: 0.63–0.86)). The other two haplotypes had low frequencies and were negatively associated with disease (Table 2). The haplotype with non-risk allele for rs3212227 and risk allele for rs7709212 showed significantly low risk (OR $=0.74$, P-value $=4.89\times10^{-2}$).

Interaction of *IL12B* with *HLA-Cw6* risk allele

HLA-Cw6 allele showed a strong (OR $=4.56$, 95% CI: 3.62–5.74) and highly significant association (P-value $<2.2\times10^{-16}$) among psoriasis patients compared with healthy controls. *HLA-Cw6* allele was present in 45.2% cases, whereas only 15.3% controls had this allele (Table 3). As *HLA-Cw6* had a much stronger effect on disease predisposition compared with *IL12B*, we wanted to determine if there was any combined effect of both these risk alleles. We considered the non-risk combination of both loci as the reference and calculated the risk imparted by different genotypes (Table 4). Presence of both *HLA-Cw6* and *IL12B* risk genotypes significantly increased the risk of disease by 9.23 (95% CI: 5.83–14.62; P-value $<2.2\times10^{-16}$) for rs7709212 and 11.68-fold (95% CI: 7.22–18.90; P-value $=<2.2\times10^{-16}$) for rs3212227 compared with the non-risk combination. Although in absence of *HLA-Cw6*, *IL12B* risk genotypes significantly increased disease risk by 1.91-fold (95% CI: 1.31–2.78; P-value $=6.83\times10^{-4}$) for rs7709212 and 2.06-fold (95% CI: 1.40–3.05; P-value $=2.32\times10^{-4}$) for rs3212227 (Table 4). When the homozygous non-risk genotype of *IL12B* was considered as reference in presence of *HLA-Cw6*, the risk still increased $\sim$1.5 times for the risk genotype (rs7709212: OR $=1.41$; rs3212227: OR $=1.54$) compared with the non-risk genotype, but was not significant at the

Table 1 Association of *IL12B* SNPs at genotype level

		Genotype frequency			P-*value*		
SNP ID	*Sample*	*TT*	*TC*	*CC*		*Age and sex adjusted*	*Adjusted (BH[a])*
rs7709212							
	Case ($N=814$)	0.4631	0.4238	0.1130	9.41×10^{-5}	3.96×10^{-5}	7.92×10^{-5}
	Control ($N=888$)	0.3773	0.4505	0.1723			
rs3212227		*TT*	*TG*	*GG*			
	Case ($N=793$)	0.4590	0.4338	0.1072	5.11×10^{-5}	6.62×10^{-4}	6.62×10^{-4}
	Control ($N=839$)	0.3766	0.4493	0.1740			

Abbreviations: BH, Benjamini–Hochberg; IL, interleukin; SNP, single-nucleotide polymorphism.
[a]Benjamini–Hochberg multiple testing correction.

Table 2 Haplotype association pattern of *IL12B* SNPs

Haplotype	*Case (freq)*	*Control (freq)*	χ^2	*Pearson's* P	*Adjusted* P-*value*[a]	*Odds ratio*	*95% confidence Interval*
CG	371.98 (0.2576)	483.57 (0.3205)	14.166	1.69×10^{-4}	3.38×10^{-4}	0.74	0.63–0.86
CT	86.02 (0.0596)	115.43 (0.0765)	3.325	6.83×10^{-2}	6.83×10^{-2}	0.76	0.57–1.02
TG	86.02 (0.0596)	119.43 (0.0791)	4.368	3.67×10^{-2}	4.89×10^{-2}	0.74	0.55–0.98
TT	899.98 (0.6232)	790.57 (0.5239)	29.759	5.01×10^{-8}	2.00×10^{-7}	1.50	1.30–1.74

Abbreviations: IL, interleukin; SNP, single-nucleotide polymorphism.
[a]Benjamini–Hochberg multiple testing correction.

level of 0.05 (Supplementary Table 4). This result indicates that irrespective of the presence or absence of *HLA-Cw6*, the OR increases 1.5–2 times for *IL12B* risk genotype compared with homozygous non-risk one (Table 4, Supplementary Table 4), and suggested lack of interaction between these two loci. Furthermore, we used multinomial logistic ratio test, including the interaction term as a covariate, which also showed no significant interaction between *IL12B* and *HLA-C* risk alleles (P-value $= 7.09 \times 10^{-1}$ for rs7709212; P-value $= 9.35 \times 10^{-1}$ for rs3212227) (Supplementary Table 5) among the psoriasis patients of India. When we classified the samples based on the presence of *HLA-Cw6*, the allele frequencies were significantly different only in *HLA-Cw6*-negative group. However, the ORs were similar between *HLA-Cw6* present and absent samples (Supplementary Table 6). The lack of significance in Cw6-positive group can be attributed to the low number of Cw6 positive individuals among the control subjects. To determine whether *IL12B* and *HLA-Cw6* had any joint functional effect, we further determined the expression pattern of the *IL12B* and its effect in the absence or presence of *HLA-Cw6* risk allele.

Over expression of *IL12B* gene in risk allele carriers

Genetic associations of *IL12B* SNPs are evident from our study. To establish the functional effect of the risk alleles, we studied the expression pattern of *IL12B* gene in the involved and uninvolved skin tissues of psoriasis patients. We performed quantitative real-time PCR from mRNAs isolated from uninvolved and involved psoriatic skin. We observed significantly high levels of *IL12B* mRNA expression in lesional skin compared with the uninvolved skin (fold change = 19.80, P-value $= 3.77 \times 10^{-7}$) (Figure 1a). We also observed significantly higher expression level of *IL12B* in patients carrying the homozygous risk genotype compared with those with homozygous non-risk genotype (rs7709212: P-value = 0.02) (rs3212227: P-value = 0.05) (Figures 1b, c). As our genetic association study showed that the *IL12B* risk haplotype was the most significantly associated haplotype with the disease, we sought to determine the expression pattern of *IL12B* gene with this risk haplotype, which again had a similar pattern but did not reach the level of significance at 0.05 (P-value $= 6.80 \times 10^{-2}$) (Figure 1d).

Considering this aspect, we determined the protein expression in the serum of psoriasis patients harboring different genotypes. We observed significantly increased IL12 serum protein concentration for both risk genotypes (for rs7709212: P-value $= 4.12 \times 10^{-5}$; rs3212227: P-value $= 1.98 \times 10^{-4}$) (Figures 2a and b), as well as with the risk haplotype (P-value $= 1.98 \times 10^{-4}$) (Figure 2c). Serum protein concentration of IL12 was significantly increased with the risk genotype compared with non-risk genotypes irrespective of the presence of *HLA*-Cw6 allele (Figures 2d and e). Furthermore, patients with the risk haplotype had significantly higher IL12 protein concentration in serum compared with the non-risk haplotype carriers both in the presence (P-value $= 3.47 \times 10^{-4}$) and absence (P-value $= 3.24 \times 10^{-3}$) of *HLA-Cw6* allele (Figure 2f). Absence of epistatic interaction between *HLA-Cw6* and *IL12B* is thus evident in terms of IL12 expression in the serum too. As p40 subunit (encoded by *IL12B* gene) is shared by both IL12 and IL23 cytokines, we also determined the expression of IL23 in serum of psoriasis patients. Although the expression of IL23 was higher in the risk genotype carriers compared with the non-risk genotype but was not significantly different (P-value $= 1.10 \times 10^{-1}$) (Supplementary Figure 1).

Table 3 Association of *HLA-Cw6* allele

	HLA-Cw6 frequency	*OR (95% CI)*	*P-value*
Cases (N=823)	0.4520	4.56 (3.62–5.74)	$<2.2 \times 10^{-16}$
Controls (N=855)	0.1532		

Abbreviations: CI, confidence interval; OR, odds ratio.

DISCUSSION

In the present study, we have determined the status of genetic association of *IL12B* gene in the pathogenesis of psoriasis. We have analyzed the association of two SNPs, rs7709212 located upstream (−6696 bp from transcription start site) and rs3212227 in the 3′-UTR of *IL12B* gene among the psoriasis patients in Eastern India. IL12B was found to be strongly associated with the disease; however, this association appeared to be independent of the presence of *HLA-Cw6* allele. The high similarities of genotype and allele frequencies between these SNPs suggest that they may be in strong linkage disequilibrium, but we observed relatively moderate linkage disequilibrium ($r^2 = 0.49$). Further evaluation suggested that only 70% of the samples were in concordance and the remaining had discordant genotypes for these SNPs. In association analysis at both genotype and allele level, we observed significant association for both SNPs only in the psoriasis patients without *HLA-Cw6* allele. However, further analysis suggested lack of any epistatic interaction between these two loci among the

Table 4 Combined effect of *IL12B* SNPs and *HLA-Cw6* risk factors

		HLA-Cw6			
		Absent		*Present*	
SNP	*Genotype*	*OR (95% CI)*	*P-value*	*OR (95% CI)*	*P-value*
rs7709212	CC	1[a] (Reference)	–	6.56 (3.42–12.58)	1.99×10^{-9}
	TC	1.51 (1.04–2.20)	3.04×10^{-2}	6.22 (4.01–9.64)	$<2.2 \times 10^{-16}$
	TT	1.91 (1.31–2.78)	6.83×10^{-4}	9.23 (5.83–14.62)	$<2.2 \times 10^{-16}$
rs3212227	GG	1[a] (Reference)		7.58 (3.88–14.81)	2.45×10^{-10}
	TG	1.85 (1.26–2.73)	1.65×10^{-3}	7.22 (4.58–11.38)	$<2.2 \times 10^{-16}$
	TT	2.06 (1.40–3.05)	2.32×10^{-4}	11.68 (7.22–18.90)	$<2.2 \times 10^{-16}$

Abbreviations: CI, confidence interval; IL, interleukin; OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]IL12B homozygous non-risk genotype in the absence of HLA-Cw6 was considered as reference.

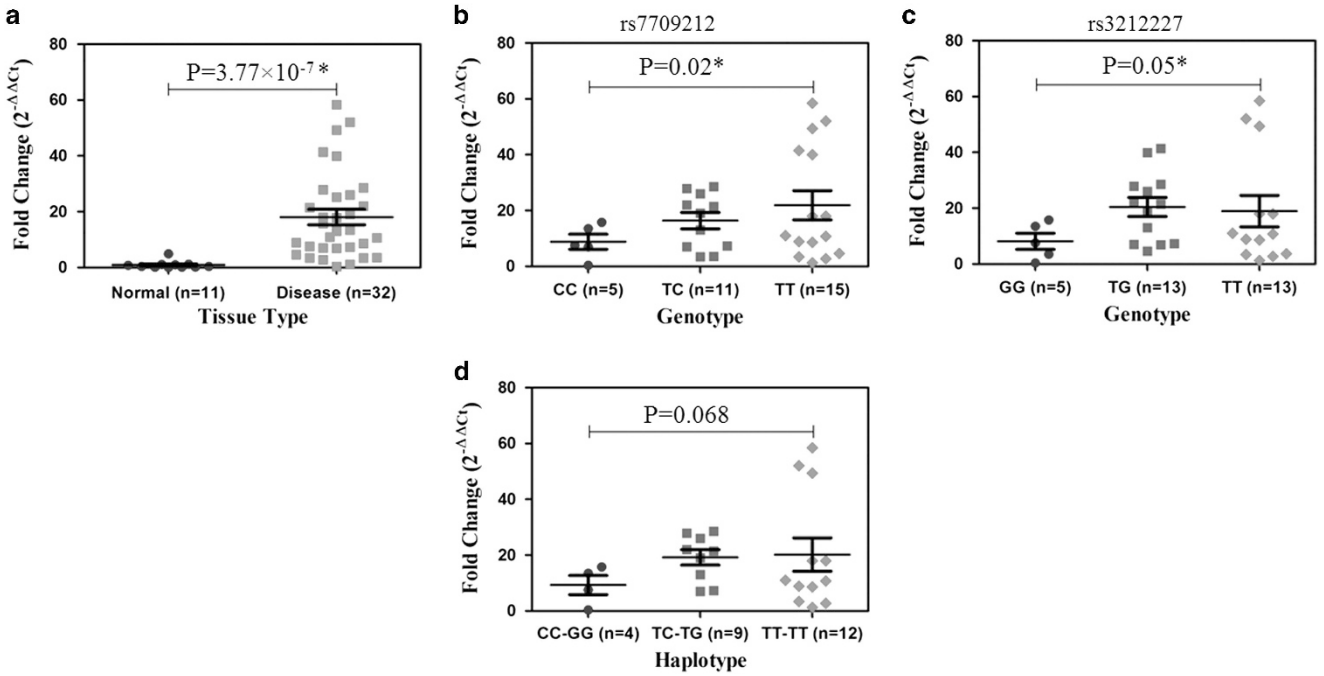

Figure 1 mRNA expression pattern of IL12B. (**a**) IL12B mRNA expression in psoriatic and adjacent normal tissue; expression with respect to genotypes of (**b**) rs7709212 and (**c**) rs3212227, and (**d**) with respect to IL12B haplotypes. **P*-value ⩽0.05 was considered significant. IL, interleukin. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

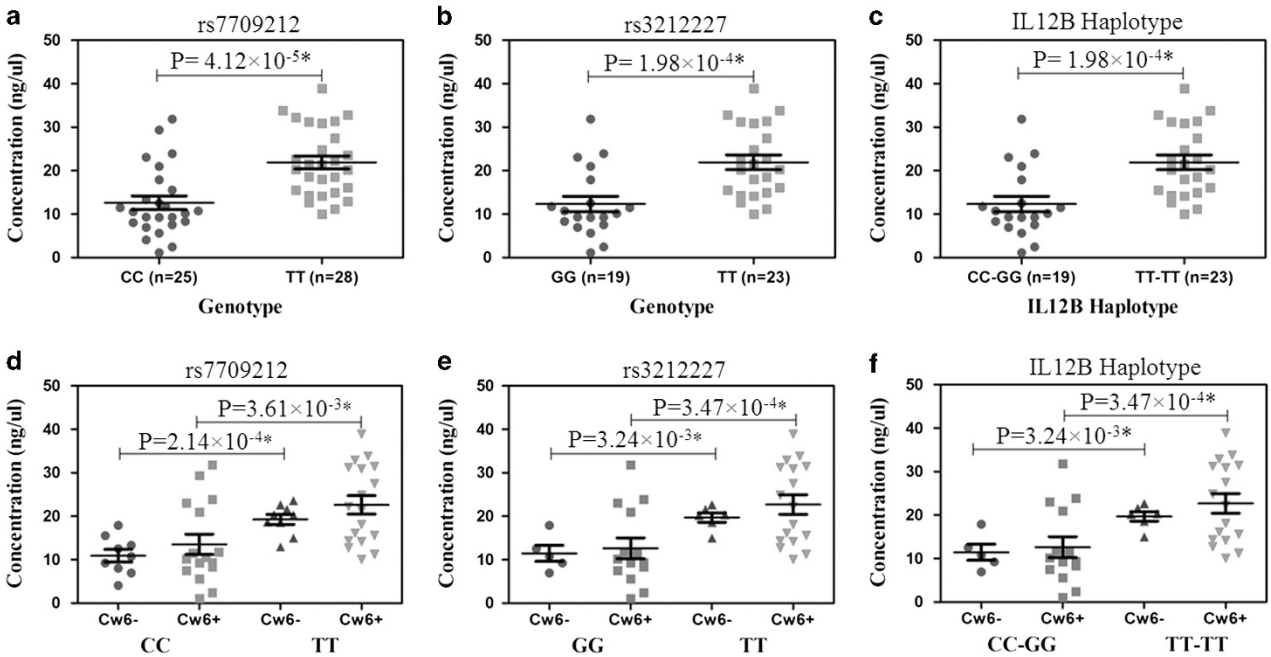

Figure 2 Expression of secreted interleukin 12 (IL12) in patient serum. Protein expression of IL12 with respect to genotype of (**a**) rs7709212 (**b**) rs3212227 and (**c**) haplotype of IL12B; protein expression after classification on the basis of absence or presence of HLA-Cw6 allele for (**d**) rs7709212 (**e**) rs3212227 and (**f**) IL12B haplotype. **P*-value ⩽0.05 was considered significant. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

psoriasis patients in India. Only 15% of the normal individuals had *HLA-Cw6* allele, and the low sample size in this group reduced the statistical power of the association analysis after *HLA-Cw6* stratification. To elucidate the functional outcome of this genetic association, we have also observed that the expression of *IL12B* gene significantly correlated with the presence of risk allele in patients. We also showed that *HLA-Cw6* also had no functional involvement in terms of *IL12* expression. Expression quantitative trait loci (eQTL) analysis in the GTEx database (http://www.gtexportal.org/home/gene/IL12B) did not find rs7709212 or rs3212227 as an eQTL for *IL12B* in blood or any

other tissue samples. Interestingly, association of rs3212227 with *IL12B* expression has also been reported in some previous studies on psoriasis,[18,26,27] suggesting that these loci might be controlling *IL12B* expression only in disease conditions. This gene, therefore, appears to be an important candidate for psoriasis and these SNPs require further functional characterization. Note that none of the SNPs studied here are listed in the eQTL database of psoriatic skin, uninvolved skin and normal skin[47] (http://csg.sph.umich.edu/junding/eQTL/).

Genetic association of *IL12B* SNPs observed in our study is in agreement with reports from south Indian population as well as with other Asian and Caucasian populations studied. This indicates that *IL12B* might be playing a prominent role in psoriasis pathogenesis. However, contradictory reports from The Netherlands and Mexican population point out to the inherent genetic variations and highlights the importance of population-specific studies.

Presence of epistatic interaction between *HLA-Cw6* and *IL12B* was debatable due to conflicting reports from Caucasian[21] and Chinese studies.[43] Interestingly, in our study, when we considered the non-risk combination (*HLA-Cw6* absent and *IL12B* homozygous non-risk genotypes) as reference, there was 9–11-folds increased risk in the presence of both risk alleles (*HLA-Cw6* present and *IL12B* homozygous risk genotypes) (Table 4). However, further analysis proved this to be only the effect of *HLA-Cw6*. In the presence of *HLA-Cw6*, there was no significant increase in risk (P-value $= 2.98 \times 10^{-1}$ for rs7709212 and P-value $= 2.02 \times 10^{-1}$ for rs3212227) between *IL12B* homozygous non-risk to risk genotypes (Supplementary Table 4). When classified based on *HLA-Cw6* status, both Cw6-positive and negative groups showed similar increase in risk due to *IL12B* risk genotype. *HLA-Cw6* status also did not have any effect on serum IL12 concentrations. It is thus evident that risk imparted by *IL12B* to psoriasis predisposition is independent of *HLA-Cw6* status in Indian population.

IL12B, encoding the p40 subunit shared between IL12 and IL23, has a prominent role in psoriasis pathogenesis is thus evident from recent studies. This fact is also supported by recent observations that a human monoclonal antibody against the p40 subunit, ustekinumab, was effective in treatment of chronic plaque psoriasis in a phase III placebo-controlled study.[48] However, a recent study on Italian population did not find any association between *IL12B* SNP genotypes and response to ustekinumab.[49] But in the presence of *HLA-Cw6*, there was a significant increase in the percentage of responders.[49] Better response in *HLA-Cw6*-positive patients from ustekinumab treatment was also observed in a Chinese population,[50] in agreement with the epistatic interaction observed in Chinese cohort.[43] However, due to the observed absence of interaction among Indian population, association of *HLA-Cw6* with ustekinumab response must be studied thoroughly before applying it in clinical practice.

In conclusion, our study showed a significant association of *IL12B* SNPs with psoriasis. Gene expression as well as serum protein concentration also varied with *IL12B* genotypes, suggesting that this gene might be playing a prominent role in psoriasis pathogenesis. However, lack of epistatic interaction with *HLA-Cw6* indicates that risk imparted by *IL12B* to psoriasis predisposition is independent of *HLA-Cw6* status in Indian population.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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