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Genetic Evidence for Involvement of the IL23 Pathway in Thai Psoriatics

Rajan P. Nair^{1,*}, Philip E. Stuart^{1,*}, Preya Kullavanijaya², Prisana Kullavanijaya², Trilokraj Tejasvi¹, John J. Voorhees¹, and James T. Elder^{1,3}

¹ Department of Dermatology, University of Michigan, Ann Arbor, MI, USA ² Institute of Dermatology, Bangkok, Thailand ³ Department of Dermatology, Ann Arbor VA Health System, Ann Arbor, MI, USA

Abstract

A recent genome-wide association analysis of psoriasis identified *IL12B* and *IL23R* as significantly associated with psoriasis. Here we report association test results of a Thai cohort consisting of 206 psoriasis cases and 114 controls. The *IL23R* SNPs rs7530511 and rs11209026, and *IL12B* SNPs rs3212227 and rs6887695 were genotyped using Taqman assays. Data were analyzed using a logistic regression model for linear trend of association. One of the *IL23R* markers, rs7530511, was marginally significant ($p=0.017$). The other *IL23R* marker, rs11209026, was not polymorphic. One of the *IL12B* markers, rs3212227, showed significant association with psoriasis ($OR = 1.64$, $p = 0.0058$) while the other, rs6887695, did not ($OR = 1.29$, $p = 0.12$). Haplotype analysis of the two *IL12B* SNPs yielded highly significant association ($p=0.00081$, $OR=1.73$). These results show that *IL12B* is an important genetic factor in psoriasis pathogenesis in the Thai population, with an association strong enough to yield significant confirmatory evidence using a modest sample size. Together with previously reported evidence for *IL12B* association in Caucasian, Japanese and Chinese psoriatics, our results support the hypothesis that genes encoding components of the IL23-mediated inflammatory pathway are important determinants of psoriasis pathogenesis across multiple racial groups.

Keywords

Psoriasis; genetic association; *IL12B*; *IL23*; Thailand; Asia

INTRODUCTION

Psoriasis is a common autoimmune inflammatory skin disease with a multifactorial genetic basis. Genome-wide linkage analyses have identified at least nine psoriasis susceptibility loci in various populations [12]. However, attempts to confirm and fine map these linked loci by linkage and association techniques have yielded reproducibility only for *PSORS1* in the major histocompatibility complex (MHC). We showed that *HLA-Cw6* is most likely the disease allele at *PSORS1* [10]. However, *PSORS1* accounts for less than 50% of the familial clustering of psoriasis [16], implying that non-MHC loci remain to be identified. In an effort to identify these loci, Cargill et al. performed a genome-wide association analysis using gene-centric markers, and identified two associated genes: *IL12B* and *IL23R* [2]. For *IL12B*, which encodes

Correspondence: Dr. Rajan P. Nair, Department of Dermatology, University of Michigan, 3430 CCGC, Box 5932, 1500 East Medical Center Dr., Ann Arbor, MI 48109-5932, USA, rnair@umich.edu, Telephone: +1 734-764-4535, Fax: +1 734-763-4575.

*These authors contributed equally to this work.

the p40 subunit common to the interleukins (IL)-12 and IL-23, the reported association was with the major allele (A-G) of a haplotype defined by SNPs rs3212227 (in the 3' untranslated region) and rs6887695 (located 60 kb upstream of the gene). For *IL23R*, the gene encoding a subunit of the IL-23 receptor, the major allele (C-G) of the haplotype defined by exonic SNPs rs7530511 (L310P) and rs11209026 (Q381R) was disease-associated. The association of *IL12B* with psoriasis was originally reported in a small Japanese study [17]. Associations of *IL12B* and *IL23R* with psoriasis have recently been confirmed in additional Caucasian cohorts [1,8,11,13] and in a Chinese cohort [4]. Together with emerging immunologic and pharmacologic data, these findings support an IL23-mediated inflammatory pathway in psoriasis pathogenesis involving IL-17-producing T-cells [7]. In this study we explored genetic association of psoriasis with *IL12B* and *IL23R* in a Thai cohort.

MATERIALS AND METHODS

The study samples were collected at the Institute of Dermatology clinic in Bangkok. The case sample consisted of 206 psoriasis cases of Thai ancestry (average and median age at onset: 34 years, age range: birth–81 years, 58% male, 42% female) diagnosed by a single experienced dermatologist (Preya Kullavanijaya). The controls were 114 individuals of Thai ancestry with no family history of psoriasis, collected from the same geographic area as the cases (average age at sampling: 45 years, median age: 44 years, age range: 16–78 years, 42% male, 58% female). All samples were obtained after written informed consent following principles of the Declaration of Helsinki. DNA was prepared from whole blood using previously established methods [9]. The *IL23R* SNPs rs7530511 and rs11209026, and *IL12B* SNPs rs3212227 and rs6887695, were genotyped using Taqman assays (Applied Biosystems, Foster City, CA). Data were analyzed for association using logistic regression and p-values were determined by Monte Carlo simulation, as described previously [11]. In all cases, the extra fit afforded by a two-term genotype regression model was not statistically significant, so only results for the simpler one-term linear trend model are discussed here. Posterior probabilities of each 2-SNP *IL12B* haplotype pair for each individual, which served as weights in regression analysis, were estimated separately for cases and controls by version 2.1 of PHASE [14,15] with default settings. Confidence intervals for risk allele frequencies were combined over sample strata based on the F-distribution with a rescaling factor [18]. Two weighting schemes were used to combine strata: equal weights and weights proportional to strata sample sizes. Power calculations were carried out with version 3.1 of the G*Power [5] using Fisher's exact test of allelic association, which is essentially equivalent to the exact ordinal logistic regression test reported here.

RESULTS

None of the SNPs had genotype frequencies that deviated significantly from Hardy-Weinberg equilibrium in controls (exact test $p > 0.6$). One of the *IL23R* markers, rs7530511, had only four occurrences of the minor allele but association with the major allele (C) was still marginally significant ($p=0.017$, Table 1). The other *IL23R* marker, rs11209026, was not polymorphic in our sample. The *IL12B* marker rs3212227 showed significant association with psoriasis (OR = 1.64, $p=0.0058$) while the other, rs6887695, did not (OR = 1.29, $p = 0.12$). Haplotype analysis of the two *IL12B* SNPs yielded strongly significant association (global $p=0.00086$), with the associated A-G haplotype ($p = 0.00081$, OR = 1.73) being the same as that found in Caucasians (Table 2). Interestingly, frequencies of the *IL12B* risk alleles and haplotype in Thais and other Eastern Asians are much lower than in Caucasians, whereas the frequencies of *IL23R* risk alleles and haplotype are substantially higher (Table 3). Similar results were seen when strata were weighted equally rather than in proportion to their sample size (data not shown). These frequency differences hold true for both cases and controls, which results in an effect size for *IL12B* SNP rs3212227 in Thais (OR = 1.64, 95% CI = 1.14–2.38)

that is similar to that in Caucasians (meta-OR = 1.48 and meta-95% CI = 1.37–1.60; for data from Cargill et al. [2], Nair et al. [11], Capon et al. [1], Smith et al. [13]). On the other hand, the effect size for *IL23R* SNP rs7530511 in Thais (OR = 9.77, 95% CI = 1.21–∞) appears larger than for Caucasians (meta-OR = 1.43, 95% CI = 1.33–1.53), but a much larger sample is needed to robustly estimate this parameter in the Thai population. Although our study provides confirmatory evidence for the association of these two SNPs in Thais, much larger samples would be required to independently establish significance at a genome-wide level. For *IL12B* SNP rs3212217, a sample size of approximately 1,900 each of cases and controls provides 80% power to detect association for a type I error rate of 1×10^{-7} , assuming a population frequency of 0.51 for the risk allele and an OR of 1.50. For *IL23R* SNP rs7530511, assuming a risk allele frequency of 0.98, enormous samples in excess of 35,000 each of cases and controls would be needed to attain genome-wide significance if the effect size is similar to that of Caucasians (OR = 1.43), and even at a very large OR of 10 (the imprecise estimate of our sample), 3,100 each of cases and controls would be required.

DISCUSSION

Our results show that *IL12B* is an important genetic determinant of psoriasis pathogenesis in the Thai population, with an effect robust enough to yield significant confirmatory evidence of association in a relatively small sample. *IL23R* also showed modest evidence of association. Much larger samples would be necessary to establish genome-wide levels of significance for the association of these two genes with psoriasis in the Thai population. Together with previously reported evidence for *IL12B* association in Caucasian, Japanese and Chinese psoriatics, our results support the hypothesis that genes encoding components of the IL-23-mediated inflammatory pathway are important determinants of psoriasis pathogenesis across multiple racial groups, despite significant differences in the prevalence of the risk alleles for *IL12B* and *IL23R* in these different populations. Resequencing these loci and/or genotyping additional closely spaced markers followed by haplotype analysis will be required to precisely define the disease-predisposing allele(s).

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Table 1

Association of *IL12B* and *IL23R* SNPs in Thai psoriatics.

Gene	SNP	Genotype ^a	Frequency in		OR (95% CI) ^b	P ^c
			Cases	Controls		
<i>IL12B</i>	<i>rs3212227</i>	AA	0.363	0.255	1.64 (1.14–2.38)	0.0058
		AC	0.517	0.509		
		CC	0.119	0.236		
	<i>rs6887695</i>	GG	0.393	0.289	1.29 (0.94–1.79)	0.12
		GC	0.408	0.474		
<i>IL23R</i>	<i>rs7530511</i>	CC	0.199	0.237	9.77 (1.21–∞)	0.017
		CC	1.000	0.965		
		CT	0.000	0.035		
	<i>rs11209026</i>	TT	0.000	0.000	—	—
		GG	1.000	1.000		
		GA	0.000	0.000		
		AA	0.000	0.000		

^aFor all SNPs, the homozygous risk genotype is given first.

^bOdds ratios and their 95% confidence intervals for an ordinal logistic model, which are calculated by permutation without recourse to asymptotic assumptions. A median unbiased estimate of the OR for SNP *rs7530511* is provided because its conditional maximum likelihood estimate is infinite.

^cMonte Carlo p-value (based on 10,000 permutations) for likelihood ratio test of linear trend of association using an ordinal logistic regression model.

Table 2Association of two-marker haplotypes for the *IL12B* region in cases and controls.

	Global $P^a = 0.00086$			
	Frequency in			
Diplotype ^b	Cases	Controls	OR (95% CI) ^c	P^d
AG/AG	0.279	0.132		
AG/other	0.469	0.487	1.73 (1.25–2.39)	0.00081
other/other	0.252	0.381		
CC/CC	0.085	0.132		
CC/other	0.414	0.411	0.81 (0.58–1.15)	0.24
other/other	0.501	0.457		
CG/CG	0.005	0.000		
CG/other	0.163	0.306	0.49 (0.29–0.82)	0.0061
other/other	0.832	0.694		
AC/AC	0.005	0.019		
AC/other	0.208	0.231	0.78 (0.48–1.26)	0.31
other/other	0.788	0.751		

^a Global P -value for Wald test of association of psoriasis and all biallelic diplotypes calculated using a weighted logistic model for linear trend of association. P -value was determined by first generating a set of 10,000 permutational test statistics by randomly shuffling the case and control labels, and then fitting a gamma distribution to the set of permutation test statistics and evaluating its cumulative distribution function.

^b In each case, the homozygous diplotype for the haplotype being tested is given first; haplotype consists of SNPs *rs3212227* and *rs6887695*.

^c Odds ratios and their 95% confidence intervals are for the weighted logistic model for linear trend of association, with a robust variance estimator.

^d P -value for Wald test of association of psoriasis and diplotype using a weighted logistic model for linear trend of association. P -values were determined by simulation as described in footnote *a*.

Table 3
Frequencies of risk alleles and haplotypes for *IL12B* and *IL23R* among Caucasians, Thais, and Eastern Asians.

Gene	SNP	Risk allele	Psoriasis Affection	Caucasian		Thai		Eastern Asian	
				No. of indiv./strata ^a	Frequency (95% CI) <i>b</i>	No. of indiv. <i>a</i>	Frequency (95% CI) <i>b</i>	No. of indiv./strata ^a	Frequency (95% CI) <i>b</i>
IL12B	rs3212227	A	case	4163/7	0.851 (0.843–0.858)	201	0.622 (0.574–0.668)	313/2	0.617 (0.577–0.655)
			control	8931/8	0.799 (0.793–0.805)	106	0.509 (0.443–0.576)	399/4	0.540 (0.504–0.575)
	rs6887695	G	case	3365/5	0.754 (0.744–0.764)	206	0.597 (0.549–0.643)	—	—
			control	6421/6	0.683 (0.675–0.691)	114	0.526 (0.462–0.590)	90/2	0.611 (0.534–0.683)
	haplotype	AG	case	2752/4	0.719 (0.707–0.731)	201	0.510 (0.461–0.559)	—	—
			control	3473/5	0.637 (0.626–0.649)	106	0.373 (0.310–0.440)	89/2	0.466 (0.391–0.543)
	rs7530511	C	case	3329/5	0.895 (0.887–0.902)	206	1.000 (0.989–1.000)	—	—
			control	5130/14	0.866 (0.858–0.872)	114	0.982 (0.954–0.995)	335/21	0.978 (0.950–0.982)
IL23R	rs11209026	G	case	4183/7	0.954 (0.949–0.958)	206	1.000 (0.989–1.000)	—	—
			control	7362/16	0.933 (0.928–0.937)	109	1.000 (0.979–1.000)	765/22	0.999 (0.985–0.999)
	haplotype	CG	case	2777/4	0.842 (0.832–0.851)	206	1.000 (0.989–1.000)	—	—
			control	3649/13	0.791 (0.781–0.800)	109	0.982 (0.952–0.995)	334/21	0.975 (0.946–0.980)

^aNumber of individuals and strata (samples) for the combination of marker, affection and race. Genotypes for Caucasians of European descent were drawn from this study and other case-control studies [1] [2] [11] [13], as well as from the CEU sample of the HapMap project [6] and eight European populations sampled by the Human Genome Diversity Project (HGDP) [3]. The discovery sample of Cargill et al. [2] was excluded to avoid biasing estimates due to the winner's curse. Genotypes for Thai are from this study, and those for Eastern Asians are from other case-control studies [4] [17] [19], as well as the HCB and JPT samples from HapMap and 19 qualifying population samples from the HGDP.

^bMean frequency and 95% confidence interval of the risk allele, combined across strata using the F-distribution with a rescaling factor [18]; weights were proportional to stratum sample size.