Association of Interleukin 23 Receptor Variants with Psoriatic Arthritis

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ABSTRACT. ***Objective.*** A recent genome-wide pooling study noted a significant association of interleukin 23 receptor (IL-23R) and psoriasis. Overxpression of IL-23 has been detected in lesional psoriatic skin, and induces epidermal proliferation. Given the interplay between psoriasis and PsA, we examined the association of IL-23R variants in 2 independent Canadian Caucasian cohorts of patients with psoriatic arthritis (PsA).

Methods. We examined 496 PsA probands and 476 controls. Cases and controls were genotyped for a panel of 11 single-nucleotide polymorphisms (SNP) in IL-23R. Allele and haplotype associations were calculated using WHAP software. P values for haplotype associations were calculated using a permutation test.

Results. The 381Gln allele of the coding SNP Arg381Gln (rs11209026) was found to be protective in the Canadian population (p = 0.004; corrected p = 0.044). A 2-marker haplotype from SNP rs7530511 and rs11209026 was associated with PsA (p = 0.011). All 3-marker sliding windows containing SNP rs11209026 were associated with PsA (p = 0.02 for all 3 windows). The magnitude of effect of IL-23R association in PsA appears to be similar to that reported in uncomplicated psoriasis.

Conclusion. Significant associations between Arg381Gln SNP and haplotypes encoding this variant were noted in PsA. It remains to be determined what contribution of this association, if any, is specifically due to the inflammatory arthritis (PsA) rather than psoriasis. (First Release Dec 1 2008; J Rheumatol 2009;36:137–40; doi:10.3899/jrheum.080458)

Key Indexing Terms:

PSORIATIC ARTHRITIS | PSORIASIS | INTERLEUKIN 23R
GENETIC ASSOCIATION | Arg381Gln | SINGLE-NUCLEOTIDE POLYMORPHISM

Psoriatic arthritis (PsA) is an inflammatory arthritis associated with psoriasis that occurs in up to 30% of patients with psoriasis[1]. Epidemiological and immunogenetic studies demonstrate a strong genetic predisposition to PsA[1]. Various cytokines including tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1), and IL-10 are postulated to play a pivotal role in the pathogenesis of PsA[1,2]. Specifically, a recent metaanalysis of TNF-α promoter polymorphisms in PsA demonstrated a significant association with the –238 variant[3]. The IL-1 gene cluster has also been found to be associated with PsA in multiple populations[4,5].

There is accumulating evidence for the role of IL-23 in psoriasis. A recent genome-wide pooling study noted a significant association with a 2-single-nucleotide polymorphism (SNP) haplotype in the IL-23 receptor (IL-23R) on chromosome 1p31[6]. One of these variants, a nonsynonymous SNP (Arg381Gln; rs11209026), is also associated with Crohn's disease, as the 381Gln allele was found to be protective in that population[7]. In addition, overexpression of IL-23 has been detected in lesional psoriatic skin, and IL-23 has been shown to induce marked acanthosis (epidermal proliferation)[8,9]. Finally, a randomized controlled trial has reported that a human IL-12/23 monoclonal antibody can substantially improve psoriasis[10].

At present there are no data regarding the genetic association of IL-23R variants and PsA. Given the interplay between psoriasis and PsA, and the overlap between PsA and Crohn's disease, we set out to examine the association of IL-23R variants among Canadian patients with PsA.

MATERIALS AND METHODS

Specimens were collected from patients with PsA with informed consent. In total we examined 496 PsA probands and 476 controls from Canada. The populations were collected from 2 major Canadian centers investigating the genetics of PsA. All patients and controls were Caucasians, mostly of North European ancestry. Two hundred forty-seven PsA probands (125 men and 122 women) and 228 controls were from Newfoundland. The mean age at entry to study for the PsA probands was 51.2 years (SD 11.5) and mean age at onset of psoriasis was 29.5 years (SD 14.5). Two hundred forty-nine PsA

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probands were from Toronto (150 men, 99 women) as well as 248 controls. The mean age at time of study for the PsA probands was 50.8 years (SD 12.6) and the mean age at the onset of psoriasis was 26.9 years (SD 11.4). Information was collected systematically and included age at onset of psoriasis and PsA, and disease pattern.

Control subjects were also obtained from the 2 sites, and were of similar ethnicity to the PsA cases from that region. Two hundred twenty-eight controls for the Newfoundland population were volunteers from Newfoundland who participated as a result of a local campaign seeking population-based controls for genetic studies. Two hundred forty-eight controls from the Toronto population were ascertained from the local HLA laboratory DNA bank that includes healthy volunteers and organ donors.

Approval for this study was obtained from the local ethics committees of Memorial University of Newfoundland and University Health Network, University of Toronto.

Cases and controls were genotyped for a panel of 11 SNP in the IL-23R genes. The 11 SNP were rs1004819, rs7517847, rs7530511, rs10489629, rs2201841, rs11465804, rs11209026, rs1343151, rs10889677, rs11209032, and rs1495965. The Sequenom MassARRAY system (Sequenom Inc., San Diego, CA, USA) was used to genotype each study participant for 11 SNP using a single-multiplex reaction. The reaction was designed using the single base extension parameters of AssayDESIGNER 3.0. The reactions were processed following the standard protocols for iPLEX with the following exceptions; 0.15 units of taq were used for each polymerase chain reaction (PCR) amplification, the concentration of SAP in the cleanup reaction was increased by 25%, and incubation at 37°C was increased by 15 min; in addition one-half the standard volumes of iPLEX termination mix and iPLEX enzyme were used in the extension reaction. The genotypes were determined using a MassARRAY compact analyzer (Sequenom).

Analysis. A combined analysis of the 2 populations as well as individual analysis for each population was conducted. Although the populations are from geographically distinct areas [a Caucasian founder population (Newfoundland) and an admixed Caucasian population (Toronto)], the control frequencies for all SNP and the direction and magnitude of associations were similar in both populations. As a result, a combined analysis was performed; a separate analysis of the individual cohorts is also included.

For the variants to be analyzed, we required the control minor-allele frequency at each locus to be > 0.05, with R^2 value < 0.8 and D' < 1.0 between adjacent markers, based on the default settings in Haploview (Broad Institute, Cambridge, MA, USA). SNP rs11209026 and rs11465804 both had minor-allele frequencies close to 0.05, and the R^2 value between the 2 SNP was > 0.8. Despite exhibiting a lower minor-allele frequency, SNP rs11209026 was retained due to the reported prominent role of this coding SNP in the studies of psoriasis and inflammatory bowel disease[6,7].

Allelic and haplotypic associations were calculated using the WHAP software package (http://pngu.mgh.harvard.edu/~purcell/whap/)[11], which uses SNPHAP[12] to estimate haplotypes via a standard expectation-maximization algorithm. WHAP performs regression-based single-marker and haplotype-association tests. All p values shown for WHAP analyses were empirically derived by permuting the data labels 1000 times. Bonferroni correction was used to account for multiple testing in the single-marker analysis. Standard chi-square tests were used for comparing genotype frequencies between cases and controls.

Analysis of haplotypic associations was done systematically across the 10 SNP that were retained using a 3-marker "sliding window" approach. Omnibus and haplotype-specific tests were performed. In addition, 2 marker tests were done to assess if a haplotype containing SNP rs7530511 and rs11209026 was associated with PsA in our cohort; a haplotype from these 2 markers was previously associated with uncomplicated psoriasis[6].

A power calculation was conducted using the Quanto software (http://hydra.usc.edu/GxE/) for SNP Arg381Gln (rs 11209026)[12-14]. The following assumptions and parameters were included in the power calculation: minor-allele frequency for rs11209026 (0.05); sample size (500 cases and controls), minor-allele frequency of 0.05, dominant inheritance model, and the disease prevalence of PsA (1/1000). The odds ratio was then varied between 1.7 and 1.8, as the OR for SNP rs11209026 was 1.78 (1/0.56) in our study. Assuming these parameters, our study has 79% to 87% power to detect an association with an OR between 1.7 and 1.8, respectively.

RESULTS

All alleles genotyped satisfied the Hardy-Weinberg equilibrium. In the combined population, single-marker associations identified 2 markers that were significantly different between cases and controls. The 381Gln variant of the coding rs11209026 was protective in the PsA population, with an OR of 0.56 (95% CI 0.38 to 0.83; p = 0.004); the corrected p value was also significant (p = 0.04). With respect to each of the populations, SNP rs11209026 had an OR of 0.52 (95% CI 0.31 to 0.90) in the Newfoundland population, and a trend was noted in the Toronto population with an OR of 0.57 (95% CI 0.32 to 1.0; Tables 1 and 2).

The CA haplotype involving SNP (rs7530511 and rs11209026) was protective in the combined population (0.004). With respect to the individual populations, CA haplotype was protective in the Newfoundland PsA cohort (p = 0.029) and a trend was noted in the Toronto population (p = 0.053).

For the 3-marker sliding-window haplotype tests, the omnibus tests noted a significant association in all 3 marker haplotypes that involved SNP rs11209026 [(rs10489629-rs2201841-rs11209026) (p = 0.02); (rs2201841-rs11209026-rs1343151) (p = 0.02); and (rs11209026-rs1343151-rs10889677) (p = 0.02)]. The 3-marker sliding-window haplotype test failed to detect a significant association solely in the Newfoundland and Toronto cohorts, likely due to the smaller sample size in the individual cohorts.

The haplotype-specific results for these 3 markers for the combined, and individual populations are presented in Table 3. The following haplotypes were all protective in the combined population, and the direction of the association was similar in the individual populations: GTA for SNP rs10489629-rs2201841-rs11209026 (p = 0.01); TAT for SNP rs2201841-rs11209026-rs1343151 (p = 0.004); and ATC for SNP rs11209026-rs1343151-rs10889677 (p = 0.004).

DISCUSSION

We found the 381Gln variant of SNP rs11209026 in the IL-23R gene to be protective in a Canadian PsA population. This was noted in the single-marker analysis, and remained significant after being adjusted for multiple testing, using the Bonferroni correction. Additionally, the omnibus and haplotype-specific tests supported this contention in the combined population. The individual populations reported a similar trend, but this was not statistically significant, likely due to the smaller sample size. There was no difference in the allele frequency of the minor coding variant of Arg381Gln SNP among the 2 major subtypes of PsA. Three hundred twenty-nine PsA probands with polyarthritis subtype had an allele frequency of 4.5% for the 381Gln variant

Table 1. Allele frequencies of IL-23R variants in PsA cases and controls from Newfoundland (NL) and Toronto (TO).

SNP rs	Minor Allele	To Cases, n = 249	TO Controls, n = 248	NL Cases, n = 247	NL Controls, n = 228
1004819	T	0.283	0.320	0.309	0.309
7517847	G	0.458	0.453	0.435	0.440
7530511	T	0.115	0.103	0.122	0.127
10489629	G	0.480	0.457	0.461	0.483
2201841	C	0.299	0.332	0.299	0.311
11209026	A	0.040	0.068*	0.041	0.076**
1343151	T	0.347	0.344	0.305	0.340
10889677	A	0.303	0.328	0.303	0.311
11209032	A	0.315	0.301	0.335	0.316
1495965	G	0.425	0.413	0.441	0.445

* p = 0.05, ** p = 0.02.

Table 2. Odds ratio for IL-23R variants in the Toronto and Newfoundland PsA cohorts.

		Combined				Toronto				Newfoundland			
SNP rs	Minor Allele	OR	Lower CL	Upper CL	p	OR	Lower CL	Upper CL	p	OR	Lower CL	Upper CL	p
1004819	T	0.92	0.76	1.11	0.39	0.84	0.64	1.09	0.20	0.98	0.74	1.39	0.87
7517847	G	1.00	0.79	1.26	0.98	0.98	0.81	1.19	0.84	1.05	0.80	1.38	0.72
7530511	T	1.05	0.80	1.38	0.72	1.13	0.78	1.63	0.52	1.03	0.70	1.51	0.89
10489629	G	1.01	0.83	1.21	0.95	1.10	0.85	1.42	0.47	1.12	0.86	1.46	0.39
2201841	C	0.900	0.74	1.09	0.28	0.86	0.65	1.14	0.29	0.97	1.28	1.28	0.81
11209026	A	0.56	0.38	0.83	0.004	0.57	0.33	1.00	0.05	0.53	0.31	0.91	0.02
1343151	T	0.93	0.77	1.13	0.48	0.99	0.75	1.30	0.92	0.85	0.64	1.11	0.23
10889677	A	0.93	0.77	1.13	0.45	0.90	0.69	1.16	0.39	0.99	0.79	1.23	0.93
11209032	A	1.08	0.89	1.32	0.41	1.07	0.79	1.45	0.67	1.11	0.83	1.47	0.48
1495965	G	1.02	0.85	1.23	0.8	1.05	0.81	1.37	0.71	1.00	0.93	1.08	0.96

CL: confidence limit.

Table 3. 3-marker IL-23R haplotype associations in the Toronto (TO) and Newfoundland (NL) PsA cohorts.

3-Marker SNP	Specific Haplotype	Combined	TO	NL
rs 10489629–2201841–11209026	GTA	0.01	—	0.03 (P)
rs220841–11209026–134351	TAT	0.004	0.05 (P)	0.03 (P)
rs11209026–1343151–10889677	ATC	0.004	0.05 (P)	0.03 (P)

P: protective association.

(minor allele A), as compared to 3.8% for 131 patients with the oligoarthritis subtype. Similarly, there was no difference in the allele frequency of Arg381Gln SNP among PsA patients with and those without axial disease. Axial disease was defined as inflammatory back pain as well as either limited range of movement of the axial spine or radiographic changes consistent with spinal disease. In the Newfoundland cohort, 34/244 (14%) patients had axial disease, and the minor allele frequency for SNP rs11209026 (A) was 4.0% in those with axial disease and 4.0% in those without axial disease. From the Toronto population 84/249 (33%) patients had axial disease, and the minor allele frequency for SNP rs11209026 (A) was 4.0% in those with axial disease and 3.9% in those without axial disease.

Psoriasis is a common, multifactorial, T cell-mediated chronic inflammatory disease that is characterized by hyperplasia of the epidermis, and infiltration of leukocytes in the dermis and the epidermis. A recent report suggests that IL-22, a TH17 cytokine, mediates IL-23-induced dermal inflammation and hyperplasia of the epidermis (acanthosis)[9]. Further, IL-23 induced marked acanthosis with a mixed dermal inflammatory cellular infiltration that is typically seen in psoriasis[9].

Genetic association studies also implicate IL-23 in psori-

asis. A recent genecentric pooling study using over 25,000 SNP noted a significant association with IL-12B[7]. Since IL-12B encodes the IL-12p40 subunit of IL-12 and IL-23, specific SNP involving variants of these 2 cytokines and their receptors were analyzed. The common haplotype of SNP encoding two IL-23R missense mutations (rs7530511 and rs11209026) resulted in susceptibility to psoriasis. Further, in a recent genome-wide study of inflammatory bowel disease (IBD), the uncommon variant of Arg381Gln (the glutamine allele) appeared to protect against the development of Crohn's disease[8]. In that IBD study, associations were also noted with other noncoding SNP that were independent of Arg381Gln.

Finally, a recent report by Krueger, *et al* noted that a human IL-12/23 monoclonal antibody is an effective treatment against psoriasis[10]. In that double-blind placebo-controlled trial, 81% of patients with psoriasis that received 4 weekly doses of IL-12/23 monoclonal antibody 90 mg achieved a 75% improvement in the Psoriasis Area and Severity Index, compared to 2% for patients that received placebo[10].

There is now mounting evidence that the IL-23 cytokine appears to play a central role in psoriasis. We were not able to differentiate the independent contribution from uncomplicated psoriasis in our patients with PsA: the strength and direction of association between PsA and IL-23R was similar to psoriasis and IL-23R in the psoriasis study. However, it is important to document the presence and magnitude of risk for IL-23R in a PsA-specific population, as the higher heritability of PsA as compared to psoriasis suggests that there are likely additional genetic factors in patients with psoriasis that predispose to PsA. There were very few patients with coexistent IBD in the combined PsA cohorts (18 out of 496 PsA probands). As a result, we were not able to assess the association of the Arg381Gln SNP or associated haplotypes, in patients with coexistent IBD.

As the disease-associated haplotype encoding 381Gln variant of SNP rs11209026 was protective in psoriasis, Crohn's disease, and ankylosing spondylitis, this nonsynonymous variant may play an important role in the pathogenesis of these disease entities. However, in the absence of functional studies we are unable to speculate if the 381Gln allele is causative in psoriasis or PsA. It is conceivable that neighboring polymorphisms may be the causative variants, as the haplotypes containing the Arg381Gln SNP exhibit a more significant association than the SNP itself. Finally, there may be multiple risk variants in this region, as the IBD genome-scan noted residual association signals throughout IL-23R[7], and it is of interest that IL-23R resides within the PSORS7 linkage peak[15].

Regarding haplotype associations, spurious associations can occur due to multilocus effects, interlocus correlations, and variation in linkage disequilibrium architecture across different populations. The other important question raised by the haplotype association is the mechanisms behind these associations.

We detected a significant protective association between the 381Gln allele on the rs11209026 SNP in IL-23R and PsA (particularly between haplotypes encoding Arg381Gln and PsA) in our Canadian cohort of patients with PsA. These results are consistent in direction and magnitude with reports documenting the association of IL-23R variants in psoriasis and Crohn's disease[6,7].

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