

CONCISE REPORT

Association of *SEEK1* and psoriatic arthritis in two distinct Canadian populations

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Objective: To examine the relationship between SNP +39604 in *SEEK1* and psoriatic arthritis (PsA) in two distinct Canadian populations.

Methods: 103 patients with PsA and 105 ethnically matched controls from Newfoundland and 202 patients with PsA and 100 controls from Ontario were studied. Patients and controls were genotyped for SNP +39604 of *SEEK1* by time of flight mass spectrometry, using the Sequenom platform. Genomic DNA was amplified by the Dynal RELI SSO HLA-Cw* typing kit for HLA-C typing.

Results: The frequency of the minor *SEEK1*(T) allele in subjects with PsA and controls was 48.5% and 32.4%, respectively (odds ratio (OR)=2.0; $p=0.017$), in the Newfoundland population and 46.5% and 38.0%, respectively (OR=1.4; $p=0.16$), in the Ontario population. Although *SEEK1* is associated with PsA, particularly in the Newfoundland population, multivariate analysis showed that *SEEK1* does not seem to be a further susceptibility factor if the HLA-Cw*0602 status is already known. No association was noted between *SEEK1*(T) allele and onset of psoriasis, PsA, or arthritis pattern.

Conclusion: *SEEK1* is associated with PsA in the Newfoundland founder population. This association is probably due to linkage disequilibrium between *SEEK1* and HLA-Cw*0602 in this population.

As psoriasis and psoriatic arthritis (PsA) are interrelated disorders, these entities, at times, share common genetic determinants.¹ The association of the HLA-Cw*0602 allele with psoriasis and PsA is one such example. HLA-Cw*0602 has long been considered to be the major predisposing factor for psoriasis,^{1,2} and the prevalence of this allele is also increased among subjects with PsA.³ Furthermore, in both these entities, the presence of HLA-Cw*0602 predicts an earlier age of onset of psoriasis.^{4,6} However, whether the HLA-Cw*0602 allele actually represents the true psoriasis gene or is in linkage disequilibrium with the true gene has not been adequately elucidated.^{1,2} *SEEK1*, which overlaps the 6p21-23 region and is in close proximity to the HLA-C locus, was recently noted to have a strong association with psoriasis in the Swedish population.⁷ In that study of 87 subjects with uncomplicated psoriasis, the +39604 polymorphism in exon 2 of *SEEK1* was found in 66% of patients with psoriasis as compared with 22% of controls ($p=0.000001$). This polymorphism exhibited the strongest association with psoriasis and its effect was independent of HLA-Cw*0602. Because of the striking association of single nucleotide polymorphism (SNP) +39604 in *SEEK1* and psoriasis, we set out to determine the prevalence of this *SEEK1* polymorphism in two distinct Canadian populations.

PATIENTS AND METHODS

Patients

This study was approved by the local ethics committees at Memorial University of Newfoundland and the University of Toronto. Informed consent was obtained from all patients. PsA was diagnosed as an inflammatory arthritis in patients with psoriasis, in the absence of other causes for inflammatory arthritis. Information was collected systematically and included age at onset of psoriasis, PsA, and disease pattern. The control subjects were obtained from their respective regions, and were all white and unrelated.

Laboratory methods

Blood samples were collected from volunteers in EDTA anticoagulant, and DNA was extracted from peripheral blood lymphocytes using the Wizard Genomic DNA Purification Kit from Promega (Madison, WI). Subjects with PsA and controls were genotyped for the *SEEK1* polymorphism by time of flight mass spectrometry, using the Sequenom platform. The polymerase chain reaction (PCR) primers were designed using MassARRAY assay design software, version 1.3.4. Mass array assay design was as follows: PCR primer 1: ACGTTGGATGTGCAACAGAAACCATCACCC; PCR primer 2: ACGTTGGATGTATGTGCTGGGAAGCTCAGTG. The primer sequences were obtained from Integrated DNA Technologies (Coralville, IA).

For HLA genotyping, 200 ng of genomic DNA was amplified using the Dynal RELI SSO HLA-Cw* typing kit. PCR amplicons were identified by a reverse line assay using sequence-specific oligonucleotide probes. Assay results were interpreted using the pattern matching program provided by Dynal.

Statistics

Logistic regression was used to study the relationship between genotyping information and case-control status. The results are summarised as odds ratios (ORs) and significance tests. The association between age at onset of psoriasis and age at onset of PsA was examined semiparametrically.⁸ A χ^2 test was used to examine the relationship between the *SEEK1*(T) polymorphism and disease pattern.

RESULTS

One hundred and three patients with PsA (42% women) and 105 ethnically matched controls from Newfoundland were assessed. The mean (SD) age of the patients with PsA from Newfoundland was 49.6 (10.7) years, mean (SD) age of onset of psoriasis was 31.0 (14.1) years and of PsA 38.0 (10.8) years. Of the 103 patients with PsA cases who were genotyped for SNP +39604, there were 3 homozygotes for the

Abbreviations: OR, odds ratio; PCR, polymerase chain reaction; PsA, psoriatic arthritis; SNP, single nucleotide polymorphism

mutant T allele (TT), 47 heterozygotes (TC), and 53 homozygotes for wild-type C allele (CC). Of the 105 Newfoundland controls genotyped, there were 3 homozygotes for the mutant T allele (TT), 31 heterozygotes (TC), and 71 homozygotes for the wild-type C allele (CC). The frequency for the minor (T) allele for the +39604 SNP was 48.5% in the Newfoundland patients with PsA compared with 32.4% in controls (OR = 2.0; $p = 0.02$).

Two hundred and two patients with PsA (41% women) and 100 ethnically matched controls from Ontario were genotyped. The mean (SD) age of the patients with PsA from Ontario was 50.5 (13.2) years, mean (SD) age of onset of psoriasis was 26.9 (12.2) years and of PsA 32.5 (10.8) years. Of the 202 patients with PsA who were genotyped for SNP +39604, there were 17 homozygotes for the mutant T allele (TT), 77 heterozygotes (TC), and 108 homozygotes for wild-type C allele (CC). Of the 100 Ontario controls genotyped, there were 6 homozygotes for the mutant T allele (TT), 32 heterozygotes (TC), and 62 homozygotes for the wild-type C allele (CC). The frequency for the minor (T) allele for the +39604 SNP was 46.5% in the Ontario patients with PsA compared with 38.0% in controls (OR 1.4; $p = 0.16$).

A combined analysis of the Newfoundland and Ontario data demonstrated an overall association between the *SEEK1*(T) allele and PsA ($p = 0.009$). Additionally, there was no evidence for a *SEEK1*(T) allele and geographic location interaction ($p = 0.39$). Thus it cannot be established that the role of the *SEEK1*(T) allele in the incidence of PsA differs in the two populations. In separate analyses, the effect of HLA-Cw*0602 is strong in the Newfoundland population ($p = 0.0004$) but is not noted in the Ontario population ($p = 0.41$). In contrast with the *SEEK1* analysis, however, a multivariate analysis does provide evidence for an interaction between HLA-Cw*0602 and geographic location ($p = 0.02$), demonstrating that the effect of HLA-Cw*0602 on the incidence of PsA is significantly different in the two populations. Furthermore, when HLA-Cw*0602 and its interaction with location is included in a multivariate model, addition of the *SEEK1*(T) allele does not improve the model significantly ($p = 0.26$). Therefore, although *SEEK1*(T) is associated with PsA, *SEEK1* does not appear to be a further susceptibility factor if the HLA-Cw*0602 status is already known. Table 1 presents a summary of the allele frequency for the minor *SEEK1* allele (T) and the presence of the HLA-Cw*0602 allele.

Linkage disequilibrium between HLA-Cw*0602 and *SEEK1*(T) is reflected in the fact that in the Newfoundland population, 18/75 (24%) of control subjects without HLA-Cw*0602 have the *SEEK1*(T) allele, whereas 20/23 (87%) with HLA-Cw*0602 have the *SEEK1*(T) allele. The parallel numbers in the Ontario population are 38/107 (36%) versus 56/57 (98%). There is some evidence of a different level of disequilibrium in the two populations ($p = 0.03$). When we explored the genotype/phenotype correlations for the minor (T) allele compared with the corresponding wild-type variant for *SEEK1*, no differences in the age of onset of psoriasis, PsA, or pattern of arthritis were noted.

DISCUSSION

Presently debate exists about whether HLA-Cw*0602 is the causative allele in psoriasis or PsA as it is neither necessary nor sufficient to develop the disease. Thus it is prudent to investigate the role of neighbouring genes, such as *SEEK1*. At first glance, +39604 SNP in *SEEK1* may not appear to be a strong candidate for PsA susceptibility as the function of *SEEK1* is not yet known and the +39604 SNP variant does not code for any amino acid change. However a striking association was noted between SNP +39604 in exon 2 of *SEEK1* and psoriasis in the Swedish population ($p < 0.000001$).⁷ This association was independent of HLA-Cw*0602.

An efficient way to validate the importance of *SEEK1* in PsA is to study multiple case-control populations, with the expectation that any important association will be seen in all populations. We examined two complementary populations. Newfoundland is a white founder population known to exhibit homogeneity comparable to the Hutterites.⁹ An advantage in examining this population is that it may allow the detection of small to modest genetic associations as a result of an increased "signal to noise ratio". Meanwhile, the Ontario population is a well characterised heterogeneous PsA population that greatly enhances the generalisability of results.

In our study a statistically significant association was shown between *SEEK1* and PsA in the Newfoundland population alone. There was a trend for increased prevalence of *SEEK1* in the Ontario PsA population as compared with controls (46.5 v 38.0%) but this was not significant. However, there was also no evidence for a different relationship in the two separate populations, but when both populations were combined the evidence for an association was considerable ($p = 0.009$). Further analysis showed, however, that this association was not independent of HLA-Cw*0602 status. The association noted in the Newfoundland population can probably be attributed to the extended linkage disequilibrium that exists in this young founder population.

A puzzling finding in our study was the lack of association between HLA-Cw*0602 and PsA in the Ontario population, especially because previous studies from this cohort have confirmed this association.^{4, 10} The allele frequency for the Ontario controls in this study (23.5%) was higher than previously reported for Ontario controls (9–15%).^{4, 10} This group of controls and patients was different from groups previously reported and may highlight the heterogeneity within the Ontario population. A further limitation of our study is the inability to rule out the possibility that an association exists for other SNP variants in the *SEEK1* gene, as only one SNP variant was studied. Finally, we are unable to decipher the independent contribution of psoriasis and inflammatory arthritis to *SEEK1*, as all our patients with PsA had both psoriasis and inflammatory arthritis.

In conclusion, data from our study does not support the premise that the +39604 SNP in *SEEK1* is an independent genetic determinant leading to susceptibility to PsA in the Newfoundland and Ontario populations.

Table 1 Allele frequency of minor *SEEK1* and HLA-Cw*0602

	Newfoundland				Ontario			
	PsA	Controls	OR	p Value	PsA	Controls	OR	p Value
<i>SEEK1</i> (T)	48.5	32.4	2.0	0.02	46.5	38.0	1.4	0.16
HLA-Cw*0602	35.9	14.4	3.3	0.0004	28.2	23.5	1.3	0.41

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