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Association between psoriasis and polymorphisms in the *TNF*, *IL12B*, and *IL23R* genes in Spanish patients

Background & Objectives: Susceptibility to psoriasis has been associated with the HLA-C*0602 allele, although it may be affected by other polymorphisms. *Materials & Methods:* We genotyped 142 patients and 160 healthy volunteers to evaluate the possible relationship between susceptibility to psoriasis and the HLA-C*0602 allele and polymorphisms in the *TNF*, *IL12B*, and *IL23R* genes. *Results:* The frequency of the wild-type *TNF*-238, *TNF*-308, and *TNF*-1031 genotypes was greater in patients with psoriasis than in healthy volunteers, although that of the mutant *TNF*-857 genotype was higher. The only difference between psoriasis and psoriatic arthritis was TNF-857. The frequency of the HLA-C*0602 allele was higher in psoriatic patients than in healthy volunteers. No differences were observed for *IL12B* and *IL23R*. Multivariate logistic regression analysis only confirmed these associations for *TNF*-238, *TNF*-857, and HLA-C*0602. *Conclusion:* Our results support an association between susceptibility to psoriasis and *TNF* polymorphisms in the Spanish population.

Key words: psoriasis, tumour necrosis factor, *IL12B*, *IL23R*, single-nucleotide polymorphisms

Psoriasis is a chronic autoimmune inflammatory disease of the skin. Genetic analyses have identified major histocompatibility complex (MHC) haplotypes bearing the HLA-C*0602 allele as the main risk factor for psoriasis [1-3].

Tumour necrosis factor alpha (TNFα) plays an important role in the pathogenesis of psoriasis [4, 5] and several polymorphisms in the *TNF* gene have been associated with this disease. The *TNF* polymorphism at position -238 has been reported to be associated with psoriasis in Caucasians [6-8], although other authors do not confirm this association [9]. A single-nucleotide polymorphism (SNP) at position -308 (G/A) has been associated with various inflammatory conditions [10] and allele A has been associated with a lower response to anti-TNF drugs [11]. In addition, a polymorphism at -857 is believed to affect the response to anti-TNF drugs [12]. However, no studies have evaluated polymorphisms in *TNF* in Spain.

Susceptibility to psoriasis has also been associated with polymorphisms in *IL12B* (which encodes the p40 subunit common to both IL-12 and IL-23) and *IL23R* in several populations [13-15].

The aim of this study was to evaluate whether the allele HLA-C*0602 and polymorphisms in *TNF* (-238, -308, -857, -1031), *IL12B* (rs6887695 and rs3212227), and *IL23R* (rs7530511 and rs11209026) are associated with psoriasis in Caucasian Spanish patients.

Materials and methods

Experimental design

Our study included 160 healthy volunteers (controls) and 142 psoriatic patients (n = 302, all Caucasian) from the Clinical Pharmacology Service and the Dermatology Service, respectively, of Hospital Universitario de La Princesa, Madrid, Spain. The controls were unrelated healthy non-smokers with no personal or family history of psoriasis. Patients were diagnosed with moderate-to-severe psoriasis (defined by a Psoriasis Area and Severity Index $\geq$10 and/or body surface area $\geq$10%) and received treatment with systemic therapy. Thirty-three patients had psoriatic arthritis. The protocol complied with Spanish law on biomedical research and was approved by the Ethics Committee for Clinical Investigation of Hospital Universitario de la Princesa.

Sample processing

DNA was extracted from peripheral blood samples in an automatic DNA extractor (MagNa Pure® System, Roche Applied Science, USA) and quantified spectrophotometrically in a NanoDrop® ND-1000 Spectrophotometer (Wilmington, USA). Purity was also tested using a 260 nm/280 nm ratio.

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Genotyping

The *TNF* polymorphisms at -238, -308, -857, and -1031 were evaluated using conventional polymerase chain reaction (PCR) and sequencing. *Table 1* shows the primer sequences and amplified product sizes. PCR products were separated in a 3% agarose gel, purified with the GeneClean® Kit (MP Biomedicals, USA), and sequenced (Applied Biosystems, USA). The results were analyzed using Chromas v.1.45.

The polymorphisms in *IL12B* (rs6887695 and rs3212227) and *IL23R* (rs7530511 and rs11209026) were evaluated using TaqMan® probes *(table 2)*. The PCR reagents used were 12.5 μL TaqMan PCR master mix and 1.25 μL 20× SNP genotyping assay (Applied Biosystems), reaching a final volume of 20 μL by addition of an 11.25 μL DNA sample at 20 ng/μL. PCR was performed with the following steps: 60°C for 30 s, denaturation at 95°C for 10 min; 40 cycles of amplification at 92°C for 15 s, 60°C for 1 min, and 30 s, and 60°C for 30 s.

The HLA-C*0602 allele was evaluated using the INNO-LiPA reverse hybridization line probe assay with the INNO-LiPA HLA-C Typing Kit (INNO-LiPA, Innogenetics, Belgium). The results were analyzed using LIRAS (Innogenetics, Belgium).

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was estimated for all the variants analyzed. Deviations were detected by comparing the observed and expected frequencies using a Fisher exact test based on the De Finetti program (available at: http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl).

Differences in allele, genotype, and haplotype frequencies of the polymorphisms on the *TNF*, *IL12B*, and *IL23R* genes and HLA-C*0602 were determined using a corrected Pearson χ^2 test (SPSS v.15; SPSS Inc, Chicago, Illinois, USA). SNPs and haplotypes with $p<0.1$ and gender were included in a stepwise multivariate logistic regression analysis to confirm the results and expressed as the odds ratio (OR), 95% confidence interval (CI) and p value. Haplotype frequencies and association with disease were calculated using SNPStats [16].

Table 1. Primer sequences used for polymerase chain reaction.

Name	Sequence 5′→3′	Size (bp)
308F	TTCCTGCATCCTGTCTGGAA	328
238R	CAGCGGAAAACTTCCTTGG	
857F	AGGAATGGGTTACAGGAGAC	173
857R	GTCCCCTGTATTCCATACCT	
1031F	TCAGAGAGCTTCAGGGATAT	149
1031R	ACATGTGGCCATATCTCCCA	

Table 2. TaqMan® probes used for genotyping.

Gene	SNP	TaqMan® probe ref.
IL12B	rs6887695	C___1994992_10
	rs3212227	C___2084293_10
IL23R	rs7530511	C___2990018_10
	rs11209026	C___1272298_10

Results

All allele frequencies were in HWE, except for *TNF*-238, -308, and -857 in the controls ($p\leq0.001$) and HLA-C*0602 in the patients ($p\leq0.001$). In the controls, mutated homozygotes were more frequent than expected, that is, 0.094 *vs*. 0.022 for *TNF*-238, 0.1 *vs*. 0.036 for *TNF*-308, and 0.075 *vs*. 0.013 for *TNF*-857. In the patients, HLA-C*0602 was more frequent than the expected, that is, 0.473 *vs*. 0.361.

Table 3 shows the genotype and allele frequencies for the polymorphisms studied. No gender differences were found for genotype frequency. In addition, there were no differences in gender proportion in controls (50% men) or patients (56.3% men).

We confirmed the association between HLA-C*0602 and susceptibility to psoriasis; the association was more frequent in patients than in healthy volunteers (47.3% *vs*. 6.4%, $p\leq0.001$).

Patients more frequently had a wild-type -238 genotype (83.1%) than healthy volunteers (80.0%) ($p\leq0.01$) *(table 3)*.

With respect to -308, patients had a higher frequency of the wild-type genotype (82.4%) than controls (71.9%), and the AA genotype was only present in the control group ($p\leq0.001$).

Mutant -857 genotypes (CT/TT) were more frequent in patients (28.2%) than in controls (15.6%) ($p\leq0.001$), but no differences were observed in T allele frequency between the groups *(table 3)*.

The frequency of the -1031 wild-type genotype was higher in patients (63.4%) than in controls (55.0%), but this difference did not reach statistical significance ($p = 0.085$). However, the C allele was more common in controls (26.9%) than in patients (20.1%) ($p = 0.05$) *(table 3)*.

No differences were observed in the genotype and allele distribution of *IL12B* (rs6887695), *IL12B* (rs3212227), or *IL23R* (rs7530511) in patients or controls. However, the *IL23R* (rs11209026) A allele was more frequent in controls than in patients (6.9% *vs*. 2.8%, $p = 0.021$).

Multivariate logistic regression analysis confirmed the differences in genotype distribution (wild type *vs*. mutant) between patients with psoriasis and controls in the case of -238 (OR = 0.37, 95% CI = 0.14-0.97, $p = 0.044$), -857 (OR = 3.08, 95% CI = 1.55-6.13, $p = 0.001$), and HLA-C*0602 (OR = 20.62, 95% CI = 8.51-49.99, $p = 0.000$) *(table 4)*. These results show that HLA-C*0602 is the most important genetic factor as it increases the frequency of psoriasis 20-fold. Nevertheless, carrying the -857 CC genotype increases the risk of psoriasis 3-fold, and carrying -238 GG reduces the risk of psoriasis by 63%.

The most frequent *TNF* haplotype was GGCT (alleles for *TNF*-238, -308, -857, and -1031), with a frequency of 45.9% in controls and 55.6% in patients *(table 5)*. The haplotype GACT was associated with a lower risk of psoriasis than the most frequently occurring haplotype (GGCT) (OR = 0.49, 95% CI = 0.25-0.97, $p = 0.041$).

The prevalence of psoriatic arthritis in the patient group was 23.2%. When comparing genotype frequencies between patients with psoriasis without arthritis and healthy volunteers, we found the same genotype and allele frequency

Table 3. Genotype and minor allele frequencies of polymorphisms in *TNF*, *IL12B*, *IL23R*, and *HLA-C*0602* in healthy volunteers, patients overall, patients with psoriasis, and patients with psoriatic arthritis.

	Genotype	Controls (a) N = 160	Patients (b) N = 142	Patients with psoriasis (c) N = 109	Patients with psoriatic arthritis (d) N = 33	p value (χ^2)			
						a *vs.* b	**a *vs.* c**	**a *vs.* d**	**c *vs.* d**
TNF-238	GG	128 (80.0%)	118 (83.1%)	92 (84.4%)	26 (78.8%)	0.007	0.012	0.267	0.577
	GA	17 (10.6%)	22 (15.5%)	16 (14.7%)	6 (18.2%)				
	AA	15 (9.4%)	2 (1.4%)	1 (0.9%)	1 (3.0%)				
A allele		14.7%	9.2%	8.3%	12.1%	0.037	0.025	0.587	0.340
TNF-308	GG	115 (71.9%)	117 (82.4%)	89 (81.7%)	28 (84.8%)	0.000	0.003	0.131	0.673
	GA	29 (18.1%)	25 (17.6%)	20 (18.3%)	5 (15.2%)				
	AA	16 (10.0%)	0 (0%)	-	-				
A allele		19.1%	8.8%	9.2%	7.6%	0.000	0.002	0.024	0.688
TNF-857	CC	135 (84.4%)	102 (71.8%)	84 (77.1%)	18 (54.5%)	0.000	0.000	0.000	0.038
	CT	13 (8.1%)	38 (26.8%)	24 (22.0%)	14 (42.4%)				
	TT	12 (7.5%)	2 (1.4%)	1 (0.9%)	1 (3.0%)				
T allele		11.6%	14.8%	11.9%	24.2%	0.241	0.897	0.006	0.014
TNF-1031	TT	88 (55.0%)	90 (63.4%)	70 (64.2%)	20 (60.6%)	0.085	0.070	0.765	0.657
	TC	57 (35.6%)	47 (33.1%)	36 (33.0%)	11 (33.3%)				
	CC	15 (9.4%)	5 (3.5%)	3 (2.8%)	2 (6.1%)				
C allele		26.9%	20.1%	19.3%	22.7%	0.050	0.042	0.485	0.538
IL-12B (rs6887695)	GG	81 (50.6%)	67 (47.2%)	54 (49.5%)	13 (39.4%)	0.127	0.154	0.326	0.522
	GC	60 (37.5%)	66 (46.5%)	49 (45.0%)	17 (51.5%)				
	CC	19 (11.9%)	9 (6.3%)	6 (5.5%)	3 (9.1%)				
C allele		30.6%	29.6%	28.4%	33.3%	0.779	0.586	0.665	0.445
IL-12B (rs3212227)	TT	99 (63.1%)	92 (64.8%)	68 (62.4%)	24 (72.7%)	0.805	0.870	0.527	0.549
	TG	54 (34.4%)	45 (31.7%)	37 (33.9%)	8 (24.2%)				
	GG	4 (2.5%)	5 (3.5%)	4 (3.7%)	1 (3.0%)				
G allele		19.7%	19.4%	20.6%	15.2%	0.907	0.800	0.387	0.323
IL-23R (rs7530511)	CC	118 (73.8%)	102 (72.3%)	78 (72.2%)	24 (72.7%)	0.616	0.661	0.753	0.994
	CT	40 (25.0%)	35 (24.8%)	27 (25.0%)	8 (24.2%)				
	TT	2 (1.3%)	4 (2.8%)	3 (2.8%)	1 (3.0%)				
T allele		13.8%	15.1%	15.1%	15.2%	0.627	0.652	0.765	0.998
IL-23R (rs11209026)	GG	138 (86.8%)	134 (94.4%)	102 (93.6%)	32 (97.1%)	0.072	0.178	0.246	0.459
	GA	20 (12.6%)	8 (5.6%)	7 (6.4%)	1 (3.0%)				
	AA	1 (0.6%)	0 (0%)	-	-				
A allele		6.9%	2.8%	3.2%	1.5%	0.021	0.062	0.092	0.466
HLA-C*0602		9 (6.4%)	62 (47.3%)	49 (49.0%)	13 (41.9%)	0.000	0.000	0.000	0.491

Corrected Pearson χ^2 test.

differences for the SNPs studied, except for the *IL23R* (rs11209026) A allele *(table 3)*. Only the *TNF*-308 A allele, the *TNF*-857 genotype and T allele and HLA-C*0602 remain significantly different between controls and psoriatic arthritis. In addition, *TNF*-857 was the only difference between patients with psoriasis and patients with psoriatic arthritis.

Discussion

Deviation from HWE was observed in the control group for the polymorphisms -238, -308, and -857. In addition, a deviation from HWE was found for HLA-C*0602, possibly reflecting its association with the disease.

Given the deviation in the control group, we first checked the genotyping method used to rule out bias and kinship, although the results remained unchanged. Possible causes of this deviation may be small sample size, and confounding factors (e.g., age, age at onset of psoriasis, psoriatic arthritis and linkage disequilibrium [17]. Rahman *et al*. [18] also found a departure from HWE for *TNF*-308*A and *TNF*-857*T in controls, probably because of linkage disequilibrium between them [19]. In fact, the haplotype GGCT (*TNF*-238, -308, -857, and -1031) was the most frequent in controls, as reported by Sánchez *et al*. [19]. Among

Table 4. Single-nucleotide polymorphisms significantly associated with psoriasis though a multivariate logistic regression analysis.

Variables in the equation	Genotype	Odds ratio (95% CI)	p value
TNF-238	GG / GA/AA	0.37 (0.14-0.97)	0.044[a]
TNF-857	CC / CT/TT	3.08 (1.55-6.13)	0.001[a]
HLA-C*0602	NO / YES	20.62 (8.51-49.99)	0.000

Inheritance models: [a] dominant.

Table 5. Frequency of *TNF* haplotypes inferred in patients and control groups (%).[a]

Haplotype	Controls (%)	Patients (%)	OR (95% CI)	p value
GGCT	45.94	55.61	1.00	-
GGCC	14.22	11.97	0.72 (0.42-1.25)	0.25
GGTT	7.77	13.34	1.35 (0.72-2.55)	0.35
GACT	12.38	7.35	0.49 (0.25-0.97)	0.041
AGCC	8.37	5.84	0.53 (0.25-1.14)	0.11
AGCT	2.55	3.31	1.23 (0.42-3.63)	0.7
GACC	2.13	1.14	0.56 (0.12-2.61)	0.46
GATT	1.70	0.32	0.29 (0.01-6.12)	0.43
AACT	2.10	NA	0 (inf-inf)	1
GGTC	1.17	1.12	0.46 (0.04-4.89)	0.52
AACC	0.75	0	0.54 (0.06-4.89)	0.59
AGTC	0.55	0		
AGTT	0.36	NA		
GATC	NA	NA		

[a] SNP positions within a haplotype are the following: -238 G/A, -308 G/A, -857 C/T, -1031 T/C. NA: not applicable.

the 14 *TNF* promoter haplotypes resolved, GGCT was most frequent in controls and patients. In controls, GACT was overrepresented, thus decreasing the risk of developing the disease.

Deviations from HWE can increase the likelihood of a false-positive association [20]; consequently, when the frequencies of two alleles are compared between cases and controls, the likelihood of a false-positive association can be substantially augmented if homozygotes for the putative high-risk allele are more common in the general population than predicted by HWE. In contrast, the chi-square statistic can be conservative if the frequency of homozygotes for the high-risk allele is lower than predicted (HLA-C*0602 in our study).

The main limitation of our study is the deviation from HWE; therefore, results should be interpreted with caution, since the genotype distribution observed would not represent a control population. Deviations could provide an additional explanation for the association between genotype and disease; therefore, although compliance with HWE is not mandatory in association studies, the information provided is useful [17].

The MHC locus is associated with psoriasis and accounts for about 30% of the genetic risk. At least 10 chromosomal regions have been associated with psoriasis, although the only region strongly associated in all studies was HLA-C*06, which is present in 67% of patients compared with 15% in the general population [21]. In addition, HLA-C*06 is associated with early onset of the disease [22] and HLA-C*0602 is the main risk allele for psoriasis, as evidenced by several research groups [1, 2, 9]. We confirm this association in our study population. Nevertheless, the functional role of this gene is unknown, although it may be associated with the innate immune system [23].

Polymorphisms in *TNF* have been associated with both susceptibility to psoriasis and response to treatment [6, 12]. We found that the TNF-238*A allele was more common among controls than patients, in contrast to previous studies that support the association between the TNF-238*A mutant allele and an increased risk of psoriasis [2, 6, 8, 9, 18, 24-27]. Reich *et al*. [7, 8] revealed enhancement of TNF-238*A only in patients with early-onset psoriasis, suggesting that the presence of *TNF*-238 may affect age at onset. In this sense, the differences between our results and those obtained by Reich *et al*. could be due to the fact that the sample comprised a mix of early- and late-onset psoriasis. We did not include this factor in our analysis. In addition, the TNF-238*A allele was more frequently detected in male patients [8], although we did not find gender differences for genotype frequency in any of the *TNF* polymorphisms studied. On the other hand, some reports showed no association between *TNF*-238A and psoriasis. Nishibu *et al*. [28] and Tsunemi *et al*. [29] found no association between TNF-238A and susceptibility to psoriasis in a Japanese population. In Korea, Kim *et al*. [30] observed no significant differences in the *TNF*-238 genotype between patients and controls. Jacob *et al*. [24] also showed that *TNF*-238 was not associated with early-onset psoriasis in a Caucasian population.

We observed that the *TNF*-308 wild-type genotype (GG) was more prevalent in patients than in controls; therefore, it could be a risk genotype. However, this relationship disappeared in the multivariate logistic regression analysis, possibly because of linkage to *TNF*-238. Settin *et al*. [31] also found the GG genotype to be more frequent in patients than in controls. In other studies, the TNF-308*A allele was less common in patients with early-onset psoriasis than in controls [7, 18]. Knowledge of this association should alert the physician and could help to determine a patient's risk of developing psoriasis. In contrast, Mössner *et al*. [25] found TNF-308*A to be more frequent in patients. Nevertheless, Höhler *et al*. [6] and Baran *et al*. [4] did not observe significant differences in *TNF*-308 genotype distribution between psoriasis patients and controls. The lack of an association between TNF-308*A and susceptibility to psoriasis has also been observed in Japan [28, 29] and Korea [30].

Other polymorphisms in the *TNF* gene were previously associated with psoriasis. We found an association between the prevalence of psoriasis and psoriatic arthritis and *TNF*-857. In particular, the *TNF*-857*T mutant allele was more common among patients (14.8%) than among healthy controls (11.6%), although the association was not statistically significant. These frequencies were similar to those

observed by Rahman *et al*. [18]: 14.8% in patients and 8.4% in controls. Surprisingly, mutant *TNF-857* genotypes (CT/TT) were more frequent in patients (28.2%) than in healthy volunteers (15.6%), and this association was confirmed in the multivariate logistic regression analysis. To date, no association has been established between this polymorphism and psoriasis. This revealing fact may aid diagnosis.

Given that psoriatic arthritis affects approximately 6-42% of patients with psoriasis [32], it is important to identify patients at high risk of this condition. Only the *TNF*-857 genotype and T allele frequency differed between patients with psoriasis and patients with psoriasis and psoriatic arthritis; therefore, this SNP may be associated with psoriatic arthritis. In particular, we found that the *TNF*-857 CT/TT and T allele were more frequent in patients with psoriatic arthritis than in controls. This association has previously been described by other authors [5, 33]. Giardina *et al*. [33] also found that the frequency of carriers of *TNF*-857 CT/TT was higher among patients with psoriatic arthritis than controls (30% *vs*. 21%). In addition, Reich *et al*. [5] reported an association between *TNF*-857 and psoriatic arthritis, but not psoriasis.

We observed that the *TNF*-1031 mutant allele and genotypes were more frequent in controls than in patients, although this association disappeared in the multivariate logistic regression analysis. Similarly, no differences were found between cases and controls in a German population [5] or a Canadian population [18].

Recently, the results of multiple well-powered genome-wide association studies have identified several additional loci outside the MHC region that are associated with the risk of psoriasis. These include three genes involved in interleukin (IL) 23 signaling (*IL23R*, *IL23A, IL12B*) [14, 34]. *IL12B* and *IL23R* encoding components of the inflammatory pathway are important determinants of the pathogenesis of psoriasis [13-15]. However, we did not find any association between psoriasis and polymorphisms in *IL12B*, although the IL23R*A allele (rs11209026) was more prevalent in controls. Nevertheless, this association was not maintained in the multivariate analysis. Hüffmeier *et al*. [35] also studied the same four polymorphisms in a German population and reported an association between psoriasis and polymorphisms in *IL12B* and *IL23R* rs11209026. Other authors reported that rs3212227 showed a significant association with psoriasis [34, 36]. In addition, Nair *et al*. [34] observed that the polymorphism rs7530511 was associated with psoriasis, while the other, rs6887695, was not.

Polymorphisms in *IL12B* and *IL23R* have been studied in Spanish patients with other inflammatory diseases such as inflammatory bowel disease [37], multiple sclerosis, celiac disease [38], ankylosing spondylitis [39], systemic lupus erythematosus (SLE) [40] and peptic ulcer disease [41]. No association was reported in patients with inflammatory bowel disease [37]. In this sense, Sánchez *et al*. [40] suggested that polymorphisms in the *IL12B* gene may not play a relevant role in the susceptibility to or severity of SLE in the Spanish population. Moreover, the *IL12B* polymorphism rs3212227 is not involved in the susceptibility to and final outcome of peptic ulcer disease in Spanish patients [41]. However, an association was observed for *IL23R* rs11209026 and susceptibility to disease in patients with ankylosing spondylitis, multiple sclerosis, and celiac disease [38, 39].

These data probably reflect the dependence of polymorphisms on population diversity, gender differences [7] and race [42]; however, we did not find differences in the distribution of genotype and allele frequency between men and women in patients or controls. Therefore, we can conclude that polymorphisms in *IL12B* and *IL23R* are not important risk factors for psoriasis.

Early gene expression profiling can identify candidate biomarkers for predicting therapeutic outcomes of treatment regimens. Treatment with TNFα blockers has been effective in refractory psoriasis and psoriatic arthritis but there remains a subgroup of patients who do not respond to TNF inhibitors and who, paradoxically, when treated, may develop TNF-induced psoriasis. Some variants of *TNF* have been associated with response to anti-TNF treatment [11, 12]. Because of the different mechanism of action of the p40 subunit of IL12 and IL23, drugs targeting this area are an alternative treatment for patients who do not respond to TNF inhibitors [43]. Analysis of polymorphisms of *TNF, IL12B*, and *IL23R* before starting treatment can provide information about how the patient will respond, and on that basis, enable physicians to tailor therapy to each patient.

In conclusion, this study shows that HLA-C*0602 is the main genetic factor associated with psoriasis, although *TNF*-238 and *TNF*-857 are also involved in susceptibility to this disease. *TNF*-857 is also associated with psoriatic arthritis. Ours is the first study to demonstrate an association between psoriasis and *TNF*-857. Our findings must be interpreted with caution owing to the deviation from HWE. ■

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