

BRIEF COMMUNICATION

*Tunisian endemic pemphigus foliaceus is associated with desmoglein 1 gene polymorphism*MB Ayed¹, P Martel², M Zitouni³, D Gilbert², H Turki⁴, M Mokni⁵, AB Osman⁵, MR Kamoun⁶, A Zahaf⁴, S Makni³, H Masmoudi¹ and F Tron²¹Laboratoire d'Immunologie, CHU Hédi Chaker, Sfax, Tunisia; ²INSERM U519, Institut Fédératif de Recherche Multidisciplinaire sur les Peptides, Faculté de Médecine et de Pharmacie, Rouen, France; ³Laboratoire d'Immunologie, Hôpital La Rabta, Tunis, Tunisia; ⁴Service de Dermatologie, CHU Hédi Chaker, Sfax, Tunisia; ⁵Service de Dermatologie, CHU La Rabta, Tunis, Tunisia; ⁶Service de Dermatologie, Hôpital Charles Nicolle, Tunis, Tunisia

Desmoglein 1 is the target antigen and probably the initiating immunogen of the autoantibody response in pemphigus foliaceus (PF), a blistering autoimmune skin disease. We previously showed that the desmoglein 1 gene (DSG1) is polymorphic and that one of its variants is associated with the sporadic form of PF observed in France. Herewith, we report, based on a case-control analysis, that the same DSG1 polymorphism participates in susceptibility to the endemic form of PF seen in Tunisia and, thus, show that common genetic factors govern the breakage of tolerance to desmoglein 1 in different epidemiological and environmental situations.

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Pemphigus foliaceus (PF) is an autoimmune blistering skin disease characterized by superficial epidermal blisters and the production of pathogenic autoantibodies directed against desmoglein 1, a 160-kDa transmembrane desmosomal glycoprotein belonging to the cadherin superfamily.¹ Two different varieties of PF have been identified. The sporadic form occurs in western Europe and North America with a low incidence (<1 case per million per year) and equally affects males and females over 50 years old.² The endemic form is observed in different geographic regions particularly in Brazil and Tunisia, and has different epidemiological characteristics.³ In Brazil, PF is known as folgo selvagem (FS), has a high incidence (25 cases per million per year) and predominantly affects young adults with no sex predominance. In Tunisia, PF is mainly observed in females ≤35 years old, among which the incidence rate reaches 15 cases per million per year.^{2,3} Patients with sporadic or endemic PF synthesize autoantibodies with similar immunochemical properties and frequently bear the same HLA class II alleles (DRB1*0102 and DRB1*0404),^{4,5} which suggests that common susceptibility loci interact with different environmental factors to cause similar clinical manifestations.

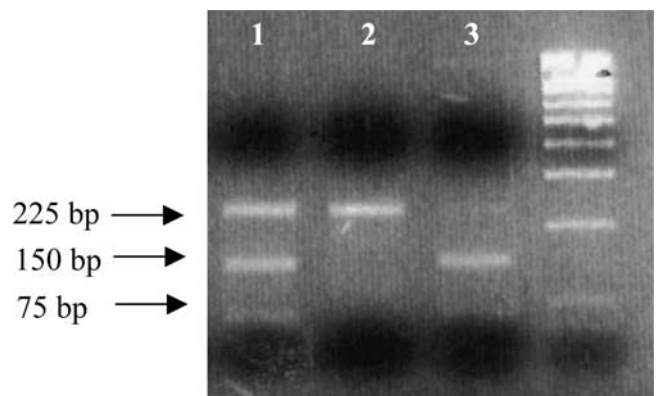


Figure 1 PCR-RFLP for genotyping of SNP (809). The procedure takes advantage of the generation of an *FauI* restriction site by the T to C substitution at position 809. Genomic DNA (50 ng) was used as the substrate for PCR amplification in a total volume of 50 μ l containing 1 μ M each of the upstream primer M809 located in intron 6 (5'-ATTTATGTAAACGTTGAGCCAAC-3') and the downstream primer EC2 (5'-GTCAATGATAATATCCCTTACATG-3'), 200 μ M each dNTP, 3 mM MgCl₂, 0.1 μ g/ μ l BSA, 1 \times *Taq* buffer and 2.5 U of *Taq* DNA polymerase. PCR procedure was as follows: 94°C 1 min, 54°C 30 s, 72°C 1 min. PCR products were incubated for 1 h at 55°C with 5 U of *FauI* (Sibenzyme, Novossibirsk, Russia) in a total volume of 50 μ l containing 1 \times SE buffer 1. Digestion products were finally electrophoresed through a 3% Agarose gel which enabled discrimination between undigested (225 bp) and digested (150 bp + 75 bp) products under UV illumination and determination of the SNP (809) genotype: C/C (complete digestion, lane 3) or T/C (uncomplete digestion, lane 1) or T/T (no digestion, lane 2).

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Table 1 Allele and genotype distributions of T/C SNP (809) in Tunisian PF patients and controls

Subjects (n)	SNP (809)*			Genotypes**		
	C (809) (n)	T (809) (n)	C (809) Allelic frequency (%)	C/C (n)	T/C (n)	T/T (n)
PF patients (49)	59	39	60.2	21	17	11
Healthy controls (141)	140	142	50.35	37	68	36

*Comparison of C (809) vs T (809) allele frequencies: $P = 0.09$; χ^2 , *l.d.f.* = 2.84, *RR* = 1.38 (95% *CI* 0.95–2). **Comparison of C/C vs C/T + T/T genotypes: $P = 0.046$, χ^2 , *l.d.f.* = 3.98, *RR* = 1.7 (95% *CI* 1.01–2.8). Allele and genotype frequencies are compared between patients and controls using the χ^2 test with Yates' correction. SPSS statistical software was used for calculation of *P* value, χ^2 , relative risk (*RR*) and 95% confidence interval (95% *CI*).

The results of our recent case-control study performed on French Caucasian patients and healthy controls showed that the desmoglein 1 gene (*DSG1*) is polymorphic and that one of its variants is associated with sporadic PF.⁶ This observation prompted us to determine whether the endemic form of PF seen in Tunisia is also associated with this polymorphic variant of *DSG1*.

Forty-nine Tunisian PF patients (10 males and 39 females; mean age 40 ± 13.5 years, range: 18–68) were recruited in the Dermatology Departments of Tunis and Sfax university hospitals, from January 1998 to December 2000. They all fulfilled the clinical, histological and immunological criteria of PF.⁷ The control population consisted of 141 Tunisian healthy blood donors (72 males and 69 females; mean age 31 years, range: 20–60).

Two polymorphic markers were previously identified in *DSG1*:⁶ a variant haplotype of five missense mutations located in exon 11, ie, on the part of the gene encoding the extracellular domains 4 and 5; and a single T to C substitution at position 809 in the region of exon 7 encoding extracellular domain 2. Only the T/C (809) single nucleotide polymorphism (SNP) was found to be involved in the sporadic PF observed in France which was associated with the presence of a cytosine at position 809 and, more strongly, with the C/C genotype of SNP (809).⁶ Thus, in the present study, we focused our analysis on the exon 7 polymorphism, using a PCR digestion technique that takes advantage of the *FauI* restriction site created by the presence of a cytosine at position 809 (Figure 1).

The distribution of the C/T (809) SNP in Tunisian PF patients and healthy controls is reported in Table 1. The allele frequencies of the C (809) were close to those observed in French PF patients (65%) and controls (48%) and, although the C (809) allele frequency was higher in Tunisian PF patients than in controls, the difference did not reach significance ($P = 0.09$). This trend prompted us to compare the distribution of SNP (809) genotypes and

especially the C/C (809) genotype in patients and controls. Significantly more patients had the homozygous C/C (809) genotype, indicating that the cytosine at position (809) SNP exerts its effect when present at the homozygous state. This finding is reminiscent of that observed in sporadic French PF,⁶ and suggests that the homozygous state facilitates the penetrance of the trait.

The demonstration that a polymorphic variant of *DSG1* is associated with the sporadic and endemic forms of PF, and the recent observation that both PF forms share common HLA class II alleles^{4,5} indicate that common genetic factors govern the breakage of B- and T-cell tolerance to desmoglein 1 in different epidemiological situations.

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