The *PRO2268* Gene as a Novel Susceptibility Locus for Vitiligo

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Vitiligo is a complex depigmentary disorder characterised by the appearance of white patches as a result of the destruction of melanocytes in the skin and hair (1). Several loci, including AIS1 (1p31) and AISI4 (4q13-q21), have been shown to confer susceptibility to the disease (2–3). However, the genetic factors involved in vitiligo are yet to be clarified. The *PRO2268* gene has been mapped, by analysis of a cDNA clone (FLB8424) from human foetal liver, to the 12q14 chromosomal region, which harbours the genes encoding interferon-gamma (IFN-γ), interleukin (IL)-26 and IL-22. Of note, the *IFN-γ-IL26-IL22* gene cluster is associated with autoimmune diseases including rheumatoid arthritis and type 1 diabetes (4–6). Although the PRO2268 protein (AF119871) has as yet unknown functions, a putative role for the *PRO2268* gene is suggested based on our unpublished observation (data not shown), by its significantly higher expression in the peripheral blood mononuclear cells (PBMCs) of vitiligo patients compared to those of control subjects ($p = 0.036$). As part of our ongoing research into the impact of susceptibility genetic variants on the risk of vitiligo, we selected the rs10784680 single nucleotide polymorphism (SNP) within the *PRO2268* gene as a candidate in our analysis.

PATIENTS AND METHODS

A cohort of 194 unaffected controls (106 females, 88 males; mean age ± SD 35.49 ± 13.82 years) with no personal or family history of vitiligo and 100 vitiligo patients (69 females, 31 males; mean age ± SD 44.74 ± 15.86 years; mean age at onset ± SD 28.69 ± 16.24 years) of Caucasian origin were enrolled into a case-control study. Diagnoses of vitiligo were made in the Department of Dermatology and Venereology, University of Tartu, based on the characteristic loss of skin pigmentation at typical locations and Wood's lamp examinations. The clinical types of vitiligo were classified as focal (one or a few macules with a nondermatomal distribution; $n = 3$), acrofacial (affecting the distal extremities and face; $n = 11$), vulgaris (scattered over the body; $n = 83$) and universal (over 90% depigmentation; $n = 3$). In addition, patients were deemed to have active vitiligo if new areas of depigmentation had been observed during the previous 3 months ($n = 74$) and stable vitiligo if no new depigmentation or enlargement of pre-existing depigmented areas had been observed for more than 3 months ($n = 26$). Moreover, vitiligo patients were categorised with respect to the presence of comorbid disorders: 38 had no other disease, 32 one or more autoimmune disorders, and 30 other diseases. The Ethics Committee of the University of Tartu approved the study and informed consent was obtained from all participants.

The rs10784680 SNP was genotyped using the SNPlex Genotyping System and analysed using a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) according to manufacturer's instructions. In brief, the assay workflow includes an allele-specific oligonucleotide reaction, exonuclease purification, and PCR amplification. The resulting amplicons are immobilised on streptavidin-coated microtitre plates. ZipChute probes are hybridised to complementary ZipCode sequences, and non-hybridised ZipChute probes and then washed away. Bound ZipChute probes are eluted and analysed by capillary electrophoresis (7).

Data analysis was performed using R software (available from www.rproject.org). Logistic regression was used to obtain odds ratio (OR) values, as well as Wald's confidence intervals (CI), for alleles and genotypes. A p value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Data analysis revealed that the frequency of the rs10784680 G allele was significantly higher in vitiligo patients (96%) than in controls (83.5%) (OR = 4.74, 95% CI 2.22–10.10; $p = 0.000055$) (Table I). Accordingly, the rs10784680 GG genotype was significantly more frequent in vitiligo patients (92%) than in controls (67%) (OR = 5.67, 95% CI 2.59–12.37; $p = 0.000013$). Notably, none of the test subjects carried the rs10784680 AA genotype. However, this observation is in accordance with the available data in the GenBank database, the rs10784680 AA genotype being reportedly absent in Hapmap-CEU, a referent population of European ancestry.

When we analysed the vitiligo patients according to their clinical subgroups, no significant differences in rs10784680 SNP allele frequency were observed when comparing patients on the basis of age at onset (> 20 years vs. ≤ 20 years; $p = 0.26$), sex (male vs. female; $p = 1$), family history (familial vs. sporadic; $p = 1$) or disease activity (active vs. stable vitiligo; $p = 0.11$) (data not shown). Furthermore, no significant differences in allele

Table I. *Allele and genotype frequencies for the rs10784680 single nucleotide polymorphism in vitiligo patients and control subjects*

rs10784680	Control *n* (%)	Vitiligo *n* (%)	OR (95% CI)	*p*-value
Allele				
A	64 (16.5)	8 (4.0)	1 (ref.)	
G	324 (83.5)	192 (96.0)	4.74 (2.22–10.10)	0.000055
Total	388 (100.0)	200 (100.0)		
Genotype				
AG	64 (33.0)	8 (8.0)	1 (ref.)	
GG	130 (67.0)	92 (92.0)	5.66 (2.59–12.37)	0.000013
Total	194 (100.0)	100 (100.0)		

OR: odds ratio; CI: confidence interval.

distribution were detected between vitiligo patients with autoimmune and other diseases and patients with no co-morbidity ($p = 0.70$ and $p = 0.63$, respectively) (data not shown). In addition, further analysis of our patients according to clinical subtype revealed that the frequency of the rs10784680 G allele was significant higher in the vitiligo vulgaris subgroup than in the control group (OR = 4.48, 95% CI 2.01–10.01; $p = 0.00024$). It should be noted that stratifying patients according to clinical groups diminished the power to detect associations.

In conclusion, to our knowledge the present study is the first to address the possible influence of the *PRO2268* gene on the risk of vitiligo. Although the function of the *PRO2268* protein remains unknown, it is of particular interest to note that the *PRO2268* gene lies adjacent to a region containing the *IFN-γ-IL26-IL22* gene cluster, whose gene products play key roles in immune signalling. For this region in particular, extensive re-sequencing, further genotyping and targeted functional studies are essential in order to identify the gene(s) that play causal roles in vitiligo. It should be noted that we cannot exclude the possibility that new susceptibility variants of the *PRO2268* gene may be discovered, or that the *PRO2268* gene may play important roles in other skin disorders.

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