

Investigation of the functional variant c.-169T > C of the Fc receptor-like 3 (*FCRL3*) gene in alopecia areata

N. Schäfer,* B. Blaumeiser,† T. Becker,‡ Y. Freudenberg-Hua,* S. Hanneken,§ S. Eigelshoven,§ C. Schmael,¶ J. Lambert,** J. De Weert,†† R. Kruse,§ M. M. Nöthen‡‡ & R. C. Betz*

Abstract

A functional variant in the Fc receptor-like 3 (*FCRL3*) gene has been implicated in susceptibility to autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus and autoimmune thyroid disease. Investigating a large case-control sample of patients with alopecia areata (AA), we found no evidence for the involvement of *FCRL3* in susceptibility to AA.

Introduction

A common genetic background for several autoimmune diseases has been demonstrated by an overlap of susceptibility alleles at different loci, including human leucocyte antigen (*HLA*) and *non-HLA* genes (Pearce & Merriman, 2006). One of these *non-HLA* regions is located on chromosome 1q21-q23, which contains a novel cluster of Fc receptor (FcR)-like receptors, also called FCRLs, FcRHs (Fc receptor homologues), IRTAs (immunoglobulin superfamily receptor translocation associated genes) or SH2 domain-containing phosphatase anchor proteins (SPAPs). These have a marked structural homology with the classical Ig Fc-receptors (FcγRs). A recent study in a Japanese sample has suggested association for a functional promoter polymorphism of the *FCRL3* gene, encoding Fc receptor-like 3 (c.-169T > C, rs7528684), and rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and auto-

immune thyroid disease (AITD, including Graves' disease [GD] and Hashimoto's thyroiditis) (Kochi *et al.*, 2005).

In the present study, we investigated whether the c.-169T > C polymorphism predisposes to the occurrence of alopecia areata (AA) in a Belgian-German sample. AA is a common skin disease presenting with patchy hair loss that affects approximately 1–2% of the general population (Safavi *et al.*, 1995). The aetiopathogenesis of AA is incompletely understood. However, AA is thought to be a tissue-specific autoimmune disease directed against the hair follicle, and association with other autoimmune diseases including AITD has been reported repeatedly (e.g. Cunliffe *et al.*, 1969; Broniarczyk-Dyla *et al.*, 1989; Puavilai *et al.*, 1994). To date, it has been postulated that various genes related to immune response are associated with AA (Tazi-Ahnini *et al.*, 2000, 2002a, 2002b; Barahamani *et al.*, 2002; Galbraith & Pandey, 1995), but only the involvement of the major histocompatibility complex (HLA) has been confirmed through independent replication (e.g. Duvic *et al.*, 1995; Colombe *et al.*, 1999; de Andrade *et al.*, 1999; Entz *et al.* in press).

FCRL3 encodes a type 1 transmembrane glycoprotein that is a member of the immunoglobulin cell surface receptor family expressed in B cells (Davis *et al.*, 2001). The c.-169T > C polymorphism in the *FCRL3* gene has been shown to alter a consensus binding site for transcription factor NF-κB, and studies of promoter activity and gel-shift binding seem to confirm a functional effect of this single nucleotide polymorphism (SNP) on NF-κB binding and on *FCRL3* transcription, with higher levels of *FCRL3* expression in B cells (Kochi *et al.*, 2005). NF-κB is known to regulate a wide variety of genes in the immune system.

Given the autoimmune component of AA and the higher incidence of AITD in patients with AA, we aimed to examine the role of the *FCRL3* gene c.-169T > C polymorphism in the development of AA by genotyping a large sample of unrelated patients and controls. Two hundred and sixty-six patients with AA (176 women and 90 men) aged 6–79 years (mean age 33.5 years) and 281 healthy unrelated blood donors with a similar sex distribution and mean age as patients were included. The patients were recruited from the outpatient hair clinics of three Departments of Dermatology: the University Hospitals of Antwerp (Belgium), Gent (Belgium) and Düsseldorf

* Institute of Human Genetics, University of Bonn, Bonn, Germany, † Department of Medical Genetics, University Hospital of Antwerp, Antwerp, Belgium, ‡ Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany, § Department of Dermatology, University of Düsseldorf, Düsseldorf, Germany, ¶ Division of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany, ** Department of Dermatology, University Hospital of Antwerp, Antwerp, Belgium, †† Department of Dermatology, University Hospital of Gent, Gent, Belgium, and ††† Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany.

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Correspondence: R. C. Betz, MD, Institute of Human Genetics, University of Bonn, Wilhelmstrasse 31, D-53111 Bonn, Germany. Tel: +49 228 287 22344; Fax: +49 228 287 2380; E-mail: regina.betz@uni-bonn.de

Table 1. Genotype and allele distributions of the *FCRL3* c.-169T > C polymorphism in controls and patients with alopecia areata (AA)

	Genotype distribution			<i>P</i> values ^a	<i>P</i> values ^b	OR (95% CI) ^c	Allele frequencies (%) ^e		<i>P</i> values ^d	OR (95% CI)
	TT	TC	CC				T	C		
Controls (<i>n</i> = 281)	79	150	52	—	—	—	54.8	45.2	—	—
Alopecia areata (<i>n</i> = 266)	90	122	54	0.595	0.199	1.12 (0.73–1.71)	56.8	43.2	0.512	1.08 (0.85–1.38)
Mild AA (<i>n</i> = 115)	33	58	24	0.588	0.068	1.16 (0.68–2.00)	53.9	46.1	0.241	0.97 (0.71–1.31)
Severe AA (<i>n</i> = 151)	57	64	30	0.731	0.068	1.09 (0.66–1.80)	58.9	41.1	0.241	1.18 (0.89–1.57)
Onset age ≤ 20 (<i>n</i> = 127)	41	63	23	0.924	0.682	1.03 (0.6–1.77)	57.1	42.9	0.531	1.10 (0.81–1.48)
Onset age > 20 (<i>n</i> = 139)	49	59	31	0.357	0.107	1.26 (0.77–2.08)	56.5	43.5	0.645	1.07 (0.8–1.43)
Family history positive (<i>n</i> = 78)	26	37	15	0.884	0.607	1.05 (0.55–1.99)	57.1	42.9	0.607	1.1 (0.77–1.57)

n indicates the number of tested individuals.

^a The frequency of the genotypes CC vs. the frequency of CT/TT using the standard χ^2 test.

^b *P* values for genotypic distributions were calculated using the Global Genotype Test.

^c The odds ratio was calculated for the CC genotype vs. the CT/TT genotypes.

^d *P* values for allelic frequencies were calculated using the Armitage Trend Test.

(Germany). Patients from the Düsseldorf hair clinic (*n* = 62) represent a group of newly diagnosed patients, while the samples from Antwerp (*n* = 171) and Gent (*n* = 33) were collected retrospectively. Clinical data were obtained from all patients, including age at onset and familial occurrence. AA type was determined according to the alopecia areata investigational assessment guidelines (Olsen *et al.*, 2004) and patients were categorized as having either patchy alopecia, alopecia totalis (AT), alopecia totalis/universalis (AT/AU), or alopecia universalis (AU). Patchy alopecia presents as one or more circumscribed patches of hair loss, AT is defined as 100% loss of scalp hair without loss of body hair, AT/AU is defined as 100% scalp hair loss with variable loss of body hair, and AU is defined as 100% loss of both scalp and body hair. The assessment of severity was based on lifetime perspective and according to the most severe episode ever experienced. The patients with AT, AT/AU and AU included in our study were classified as having severe AA, whereas the patients with patchy AA were classified as having mild AA. One hundred and fifteen patients presented with patchy AA, 25 with AT, 14 with AT/AU and 112 with AU. Seventy-eight of 266 patients reported a family history of AA (29.3%), defined as having at least one first- or second-degree relative with AA (Blaumeiser *et al.*, 2006). Patients and controls were all of Central European origin. Blood donors were not specifically screened for the absence of AA as this factor has little impact on the power of a case-control study when the investigated disease has a population prevalence of approximately 1–2%, as reported for AA (Moskvina *et al.*, 2005). Ethical approval for the study was obtained from the relevant Ethics Committees. Written informed consent was obtained before taking blood from patients with AA and controls. DNA was extracted from peripheral blood leucocytes according to standard methods. The 5' UTR of exon 2 of the *FCRL3* gene, including SNP c.-169T > C, was amplified by PCR under standard conditions, and double-strand sequencing of PCR products was performed.

Since Kochi *et al.* (2005) found the strongest evidence for association under a recessive model for the C allele, we tested the frequency of the genotype CC vs. the frequency of CT/TT using a standard χ^2 test. Additionally, genotypic distributions between cases and control subjects were compared using the Global Genotype Test and allele frequencies using the Armitage Trend Test. Power values were calculated assuming the genotype distributions reported by Kochi *et al.* (2005).

Distributions of genotypes were consistent with Hardy-Weinberg equilibrium in both groups. Our results (Table 1) do not support a significant association for the risk genotype in our patient sample with AA (*P* = 0.595). Furthermore, the analysis of subgroups of individuals with either severe AA or an early age of onset (onset age ≤ 20 years) did not reveal an association of this polymorphism (*P* = 0.731, *P* = 0.924). We also tested the subgroup of patients who had a positive family history, since a genetic effect might be stronger in familial cases where a genetic contribution to disease is more likely. No positive association emerged in this subgroup either, however (*P* = 0.884). Similarly, negative results were obtained by testing for differences in global genotype distributions and allele frequencies (Table 1).

The findings from our case-control sample do not support an association between the *FCRL3* variant c.-169T > C and AA. Inadequate power is an unlikely explanation of our data. We had sufficient power of 97.2% to detect an odds ratio (OR) of 2.15 of the CC-genotype when replicating the previously reported results for RA (Kochi *et al.*, 2005).

Given the power of our study, a likely explanation for our negative finding in patients with AA is that the *FCRL3* gene is not involved in the pathophysiology of AA, and that *FCRL3* does not constitute a genetic factor that AA shares with other autoimmune disorders such as RA, SLE, AITD and GD. Another possibility is that c.-169T > C is in LD with a functional variant that increases the risk of AA, and that differences in LD structure

between the different populations investigated account for the discrepant findings. The fact that strong associations with autoimmune diseases have been observed in the Japanese population (Ikari *et al.*, 2005; Kochi *et al.*, 2005) but no, or only weak, associations have been found in Western populations (Hu *et al.*, 2006; Martinez *et al.*, 2006; Simmonds *et al.*, 2006; Smyth *et al.*, 2006) would support this assumption. However, the functional data comprising much of the evidence presented by Kochi *et al.* (2005) depend on c.-169T > C itself being the susceptibility variant. It is also possible that c.-169C > T exerts its effect only in conjunction with environmental factors that might be present in one population but not in the other. No experimental data exist to support this hypothesis, however. Finally, it cannot be excluded that a functional variant of the *FCRL3* gene that is not in linkage disequilibrium with c.-169C > T contributes to the development of AA.

In conclusion, our study fails to support the hypothesis that the functional c.-169T > C variant of the *FCRL3* gene directly influences susceptibility to AA.

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