

BRIEF COMMUNICATION

Investigation of the p.Ser278Arg polymorphism of the autoimmune regulator (*AIRE*) gene in alopecia areata

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Abstract

A recent study has suggested that the g.961C>G (p.Ser278Arg) variant of the autoimmune regulator (*AIRE*) gene contributes to susceptibility to alopecia areata (AA). We attempted to replicate this finding using a case-control sample of Belgian-German origin (273 patients and 283 controls). Despite adequate power, our study results do not support a significant association of the risk allele in our AA patient sample. This remained the case when we stratified our sample according to severity and family history of disease. Our study results do not support the hypothesis that the g.961C>G (p.Ser278Arg) polymorphism of the *AIRE* gene is associated with an increased risk for AA.

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Alopecia areata (AA) is a common skin disease presenting with patchy hair loss that affects approximately 1–2% of the general population (1). The etiopathogenesis of AA is incompletely understood. AA is thought to be a tissue-specific autoimmune disease directed against the hair follicle and may be associated with other autoimmune diseases. The mechanism of hair follicle dysfunction is immunological and is controlled by activated T-cells. Susceptibility to AA is likely to follow that of a complex polygenic trait. To date, various genes related to immune response have been postulated to be associated with AA (2–6), but only the major histocompatibility complex (HLA) has been confirmed by independent replication (7–10).

Tazi-Ahnini and colleagues (6) recently reported an association between genetic variation in the autoimmune regulator (*AIRE*) gene and AA. There are two main reasons why the *AIRE* gene may be considered a promising candidate gene: (i) the *AIRE* protein is involved in the expression of ectopic proteins by medullary thymic epithelial cells and thereby allows the establishment of central tolerance, contributing to the prevention of organ-specific autoimmunity; (ii) homozygous loss-of-function mutations in the *AIRE* gene are responsible for the autoimmune syndrome polyendocrinopathy candidiasis ectodermal dysplasia (APECED; OMIM *240300), a rare autosomal recessive disorder (11) in which around one-third of patients demonstrate AA (12).

Tazi-Ahnini et al. (6) screened the coding sequence of the *AIRE* gene (GeneBank accession number: AB006684, mRNA) and identified 20 variants within the intron – exon junctions and in exonic regions. Two of these variants

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(g.961C>G and g.1029T>C) were observed to give rise to amino acid substitutions (p.Ser278Arg and p.Val301Ala) and were used for further association studies. With the study of 202 AA patients and 175 controls of North English origin, the g.961G allele of the g.961C>G (p.Ser278Arg) polymorphism was identified as a susceptibility factor for AA ($P = 0.0093$, OR = 1.99), the effect being attributed to severely affected patients presenting with complete hair loss [alopecia universalis (AU), $P = 0.0012$, OR = 3.27] or early onset of the disease (≤ 30 years, $P = 0.0082$, OR = 2.22). The g.1029T>C polymorphism (p.Val301Ala) showed no association with the disease. Tazi-Ahnini and colleagues (6) emphasized the potential functional relevance of the g.961C>G variant, observing that it leads to a change from a polar amino acid (serine) to a charged amino acid (arginine) and that it is localized in the SAND domain of the protein. A SAND domain is characteristic of proteins involved in chromatin-dependent transcriptional regulation (13) and likely to be relevant to DNA binding (14).

Although the data in the report by Tazi-Ahnini et al. (6) are consistent with the hypothesis that the variant they report alters susceptibility to AA, the hypothesis still requires a convincing degree of support from independent human genetic studies. We therefore sought to replicate the reported finding of the role of the *AIRE* gene g.961C>G polymorphism in the development of AA by genotyping a large independent sample of unrelated patients and controls. A total of 273 patients with AA (177 women and 96 men) aged 6–79 years (mean age 39.5 years) and 283 healthy unrelated sex and age-matched blood donors 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 (Germany). Patients from the Düsseldorf hair clinic ($n = 63$) represent a group of newly diagnosed patients, while the samples from Antwerp ($n = 177$) and Gent ($n = 33$) were collected retrospectively. Clinical data were obtained from all patients, including age at onset and familial occurrence. The AA type was determined according to the alopecia areata investigational assessment guidelines (15), and patients were categorized as having 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 was defined as 100% loss of scalp hair without loss of body hair; AT/AU was defined as 100% scalp hair loss with variable loss of body hair; AU was defined as 100% loss of both scalp and body hair. All AU patients included in our study had a history of circumscribed patches with complete hair loss and repeated times of hair regrowth. A total of 116 patients presented with patchy AA, 25 with AT, 14 with AT/AU, and 118 with AU. Ninety-two out of 273

patients reported a family history of AA (33.6%), defined as having at least one first- or second-degree relative with AA (16). Blood donors were not specifically screened for the absence of AA, as this will have little impact on the power of a case-control study when the studied disease has a population prevalence of approximately 1–2% as reported for AA (17). After obtaining written informed consent, blood was taken from AA patients and controls. DNA was extracted from peripheral blood leukocytes according to standard methods. Ethical approval for the study was obtained from the relevant Ethics Committees.

The coding region of exon 7 of the *AIRE* gene, including g.961C>G, was amplified by PCR under standard conditions, and double-strand sequencing of PCR products was performed.

As Tazi-Ahnini et al. (6) found the strongest evidence for association under a dominant model for the G allele, we tested the frequency of the genotypes gg/gc vs the frequency of cc using a standard χ^2 -test. Additionally, genotypic distributions between cases and control subjects were compared using the Armitage Trend test. Power values were calculated assuming the genotype distributions reported by Tazi-Ahnini et al. (6).

Distributions of genotypes were consistent with Hardy–Weinberg equilibrium in both groups. Our study results (Table 1) do not support a significant association of the risk allele in our AA patient sample ($P = 0.126$). Furthermore, the analysis of subgroups of individuals with AU or with an early age of onset (onset age ≤ 20 years) did not reveal an association of this polymorphism ($P = 0.211$, $P = 0.135$). The result remained negative when we used the age at onset cutoff employed in the original study by Tazi-Ahnini et al. (onset age ≤ 30 years, data not shown). We also tested the subgroup of patients with a positive family history as 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 ($P = 0.774$).

The findings from our case-control sample do not support the reported association between the *AIRE* variant g.961C>G and AA. Inadequate power is an unlikely explanation of our data. We had sufficient power (>80%) to replicate the effects reported by Tazi-Ahnini et al. (6).

A possible explanation for our non-replication is that the report by Tazi-Ahnini and colleagues (6) represents a false-positive finding and that the *AIRE* gene is not involved in the pathophysiology of AA. Another possibility is that g.961C>G is in LD with a functional variant that increases the risk of AA and that differences in LD structure between the different populations studied account for the discrepant findings. The possibility that another, truly causative, variant exists is not supported by the results of the sequencing experiments performed by

Table 1 Genotype and allele distributions of the AIRE g.961C>G polymorphism in controls and AA patients

	Genotype distribution			<i>P</i> -values ^a	OR (95% CI)	Allele frequencies		
	GG	GC	CC			G (%)	<i>P</i> -values ^b	OR (95% CI)
Controls (<i>n</i> = 283)	0	53	230	–	–	–	–	–
Alopecia areata (<i>n</i> = 273)	2	36	235	0.126	0.7 (0.45–1.11)	40 (7.3)	0.209	0.77 (0.5–1.17)
Patchy AA (<i>n</i> = 116)	2	12	102	0.106	0.6 (0.32–1.12)	16 (6.9)	0.252	0.72 (0.4–1.28)
AU (<i>n</i> = 118)	0	16	102	0.211	0.63 (0.37–1.25)	16 (6.8)	0.211	0.7 (0.39–1.26)
Onset age ≤ 20 (<i>n</i> = 85)	0	10	75	0.135	0.58 (0.28–1.19)	10 (5.9)	0.135	0.6 (0.3–1.2)
Family history positive (<i>n</i> = 92)	0	16	76	0.774	0.91 (0.59–2.03)	16 (8.7)	0.774	0.91 (0.59–2.05)

^a*n* indicates the number of tested individuals.

^aThe frequency of the genotypes gg/gc vs the frequency of cc using the standard χ^2 -test.

^bThe *P*-values for genotypic distributions were calculated using the Armitage Trend test.

Tazi-Ahnini and colleagues (6) as part of their original study, in which they detected no further variants of potential functional significance through the sequencing of exons as well as exon – intron junctions in nine unrelated individuals. However, a functional variant may reside in a regulatory region of the gene, or in the coding region of a small subgroup of patients which escaped detection due to the fact that sequencing was only performed in a limited number of individuals. It also remains possible that LD extends to a gene that resides in close vicinity to the *AIRE* gene, such as the inducible T-cell co-stimulator ligand (*ICOSLG*) gene which acts as a co-stimulatory signal for T-cell proliferation, cytokine secretion, and B-cell proliferation, and is only 45 kb apart from the *AIRE* gene. As discussed previously, if the original result was correct and an as yet undetected variant in LD with g.961C>G caused the original association, the fact that our study showed no association implies the existence of differences in haplotype structure between North of England and German/Belgian populations, a possibility that has not been explored for this chromosomal region.

Although it is an interesting potential candidate gene for a number of autoimmune diseases, no other autoimmune disorder has yet been associated with genetic variation in the *AIRE* gene. A study investigating the presence of 967–979del13bp, a common APECED mutation, found no mutation carriers amongst 90 patients with Addison's disease (18). Mutations in exon 6 and 8 of the *AIRE* gene have not been associated with inflammatory bowel disease (19).

In conclusion, our study fails to support the hypothesis that the g.961C>G polymorphism in exon 7 of the *AIRE* gene directly influences susceptibility to AA.

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