

The R620W polymorphism in *PTPN22* confers general susceptibility for the development of alopecia areata

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Summary

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Key words

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Conflicts of interest

None declared.

Background The functional R620W (c.1858C>T) variant of the protein tyrosine phosphatase nonreceptor 22 gene (*PTPN22*) has been associated with a variety of autoimmune disorders. A recent study has suggested that R620W also contributes to the severe form of alopecia areata (AA).

Objectives We sought to replicate the finding of an association between *PTPN22* and severe AA. In addition, we wanted to study the effect of *PTPN22* on the general risk to develop AA and on other subtypes of AA (mild AA, early/late age at onset, positive/negative family history).

Methods The R620W variant was genotyped in a large case–control sample of Belgian–German origin with 435 patients and 628 controls.

Results Significant results were obtained for the overall collective of patients with AA ($P = 0.007$). Subdividing the sample according to severity of AA, family history and age at onset, we detected lowest P -values for patients with the severe form of AA ($P_{\text{corr}} = 0.036$), with a positive family history ($P_{\text{corr}} = 0.042$) and with an age at onset ≤ 20 years ($P_{\text{corr}} = 0.048$).

Conclusions Our results suggest the R620W variant of *PTPN22* as a general risk factor in AA with the strongest effect observed among patients with a severe type of AA, a positive family history or an early onset of disease.

Alopecia areata (AA) is a common skin disease presenting with patchy hair loss which affects approximately 1–2% of the general population.¹ A familial occurrence of AA is well established, with recurrence risks of 5–8% in first-degree relatives of affected individuals.² The pattern of familiarity suggests that the genetic basis is multifactorial. The aetiopathogenesis of AA is not completely understood. However, AA is thought to be a tissue-specific autoimmune disease directed against the hair follicle, and association with other autoimmune diseases has been repeatedly reported.^{3–5} To date, it has been postulated that various genes related to immune response are associated with AA, but only the involvement of the major histocompatibility complex has been confirmed by means of independent replication.^{6,7}

Kemp et al. recently reported an association between the genetic variant c.1858C>T in the protein tyrosine phosphatase

nonreceptor 22 gene (*PTPN22*) and a severe form of AA.⁸ The common, functionally relevant nonsynonymous substitution c.1858C>T (R620W, rs2476601) has been previously implicated with a number of autoimmune diseases, with confirmed association for rheumatoid arthritis, juvenile idiopathic arthritis, systemic lupus erythematosus, Graves disease and type 1 diabetes mellitus.⁹ *PTPN22* (also called LYP encoding lymphoid protein tyrosine phosphatase), located on chromosome 1p13.2, has a negative regulatory effect on T-cell activation through binding to the Csk protein.^{10–13} The risk-associated W620 allele appears to lower thresholds for T-cell receptor signalling,¹¹ which may predispose individuals carrying the risk allele to autoimmunity.

In their study, Kemp et al.⁸ determined the c.1858C>T polymorphism of the *PTPN22* gene in a sample of 196 English

patients with AA and 507 healthy individuals using a case-control design. Whereas no significant differences in genotype distributions and allele frequencies between patients and controls were found in the overall sample, the authors found a significant over-representation of the CT genotype ($P = 0.006$) and the C allele ($P = 0.013$) among severely affected patients presenting with complete loss of scalp hair (alopecia totalis, AT) or of scalp and body hair (alopecia universalis, AU).

Although the data in the report of Kemp *et al.*⁸ are consistent with the view that mechanisms of autoimmunity play an important role in the development of AA, the hypothesis still requires a convincing degree of support from independent human genetic studies. We therefore sought to replicate the reported finding by genotyping a large independent sample of unrelated patients and controls. In addition, we wanted to study the effect of PTPN22 on the general risk to develop AA and on other subtypes of AA (mild AA, early/late age at onset, positive/negative family history).

Patients and methods

Four hundred and thirty-five patients with AA (292 women and 143 men) aged 5–82 years (mean 42.3) and 628 healthy unrelated sex- and age-matched blood donors were included. The patients were recruited from the outpatient hair clinics of four Departments of Dermatology, i.e. the University Hospitals at Antwerp and Gent ($n = 220$), Düsseldorf ($n = 125$) and Bonn ($n = 19$), from a private dermatology practice in Wesseling ($n = 47$) and from AA support groups ($n = 24$). Clinical data were obtained from all patients, including age at onset and familial occurrence. The AA type was determined according to the AA investigational assessment guidelines¹⁴ and patients were categorized as having either patchy alopecia, AT, AT/AU or 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, and 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. One hundred and ninety-six patients presented with patchy AA, 34 with AT, 26 with AT/AU and 179 with AU. One hundred and twenty-three of 435 patients reported a family history of AA (28.3%), defined as having at least one first- or second-degree relative with AA.² The patient group was subdivided by the age at onset using a cut-off employed by Kemp *et al.*⁸ (early disease onset, ≤ 20 years; late disease onset, > 20 years). Patients and controls were all of Central European origin. 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.¹⁵ After obtaining written informed consent, blood was taken from patients with AA and controls. DNA was extracted from peripheral blood leucocytes according to standard methods. Ethical approval for the study was obtained from the relevant Ethics Committees.

The variant c.1858C>T of the PTPN22 gene was amplified by polymerase chain reaction under standard conditions. In a second step, a restriction digest was performed. The c.1858T allele creates a XcmI restriction site which was used for screening of all patients and controls. The accuracy of the genotyping procedure was confirmed by sequencing at least 10 individuals for each genotype category.

Genotypic distributions between cases and control subjects were compared using the Armitage trend test. Allele frequencies were compared using a standard χ^2 test.

Results

The distribution of genotypes was consistent with Hardy-Weinberg equilibrium in the patient ($P = 0.468$) and control

Table 1 Frequency of the PTPN22 polymorphism c.1858C>T in controls, patients with alopecia areata (AA) and their subgroups

| | Genotype distribution (%) | | | | Allele frequency (%) | | | |
|--|---------------------------|------------|----------|----------------------|----------------------|------|----------------------|-----------------|
| | CC | CT | TT | P-value ^c | C | T | P-value ^c | OR (95% CI) |
| Controls ($n = 628$) | 506 (80.6) | 112 (17.8) | 10 (1.6) | – | 89.5 | 10.5 | – | – |
| Patients ($n = 435$) | 320 (73.6) | 104 (23.9) | 11 (2.5) | 0.007 | 85.5 | 14.5 | 0.006 | 1.4 (1.11–1.87) |
| Mild AA ($n = 196$) | 145 (74.0) | 50 (25.5) | 1 (0.5) | 0.132 (0.792) | 86.7 | 13.3 | 0.130 (0.782) | 1.3 (0.92–1.84) |
| Severe AA ($n = 239$) | 175 (73.2) | 54 (22.6) | 10 (4.2) | 0.006 (0.036) | 84.5 | 15.5 | 0.004 (0.025) | 1.6 (1.15–2.12) |
| FH+ ^a ($n = 123$) | 86 (69.9) | 33 (26.8) | 4 (3.3) | 0.007 (0.042) | 83.3 | 16.7 | 0.006 (0.034) | 1.7 (1.16–2.49) |
| FH– ^b ($n = 308$) | 232 (75.3) | 69 (22.4) | 7 (2.3) | 0.065 (0.390) | 86.5 | 13.5 | 0.059 (0.352) | 1.3 (0.99–1.78) |
| Onset age ≤ 20 years ($n = 196$) | 141 (71.9) | 49 (25.0) | 6 (3.1) | 0.008 (0.048) | 84.4 | 15.6 | 0.007 (0.040) | 1.6 (1.13–2.18) |
| Onset age > 20 years ($n = 239$) | 179 (74.9) | 55 (23.0) | 5 (2.1) | 0.076 (0.458) | 86.4 | 13.6 | 0.070 (0.421) | 1.3 (0.96–1.84) |

OR, odds ratio; CI, confidence interval; FH, family history.

^aDefined as a history of at least one first- or second-degree relative with AA. ^bDefined as no history of first- or second-degree relative with AA. ^cP-values in brackets have been corrected for multiple testing ($P_{\text{corr}} = 6 \times P_{\text{uncorr}}$).

group ($P = 0.200$). No significant differences in genotype or allele frequencies were observed between males and females in either patients or controls (data not shown). The frequency of the T allele (W620) in controls (10.5%) was similar to the frequency observed in other German control populations.^{16,17}

Analysis of genotype distribution (Table 1) suggests a significant contribution of the W620 allele to the development of AA ($P = 0.007$). The frequency of the T allele increased from 10.5% among controls to 14.5% among patients [odds ratio (OR) 1.4, 95% confidence interval 1.11–1.87]. Such a general effect on the risk to develop AA was not detected in the study by Kemp et al.⁸ This may be, however, a result of limited power as in their study a higher frequency of the T allele was also observed among patients (10.5%) compared with controls (8.5%) ($P = 0.246$). The power to significantly detect an OR of 1.4 was only 46% in the study by Kemp et al.⁸

The analysis of subgroups of patients revealed the strongest genotypic associations among patients with either severe AA (AT, AT/AU, AU) ($P_{\text{uncorr}} = 0.006$, $P_{\text{corr}} = 0.036$), early age at onset (≤ 20 years) ($P_{\text{uncorr}} = 0.008$, $P_{\text{corr}} = 0.048$) or positive family history ($P_{\text{uncorr}} = 0.007$, $P_{\text{corr}} = 0.042$). Similarly positive results were obtained by testing for differences in allele frequencies (Table 1). The results in the remaining subgroups were either borderline significant (family history negative, $P_{\text{uncorr}} = 0.065$, $P_{\text{corr}} = 0.390$) or showed a nonsignificant trend towards association (mild AA, $P_{\text{uncorr}} = 0.132$, $P_{\text{corr}} = 0.792$; onset age > 20 years, $P_{\text{uncorr}} = 0.076$, $P_{\text{corr}} = 0.458$), suggesting that smaller genetic effects probably operate among these patients.

Discussion

Our study strongly suggests that the functional R620W variant of the PTPN22 gene directly influences susceptibility to AA. The effect is driven mainly by patients characterized by a severe course of the disease, positive family history or early disease onset. The genetic data have to be complemented by functional studies in the future in order to understand how the risk-associated W620 allele contributes to the pathophysiology of AA. Available functional data obtained in PEP knockout mice, the murine homologue of PTPN22, suggest that this effect may be conferred through lowering thresholds for T-cell receptor signalling.¹¹

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