CONCISE REPORT

The association of the PTPN22 620W polymorphism with Behçet's disease

Vijay Baranathan, Miles R Stanford, Robert W Vaughan, Elli Kondeatis, Elizabeth Graham, Farida Fortune, Wafa Madanat, Charlie Kanawati, Marwen Ghabra, Philip I Murray, Graham R Wallace

Ann Rheum Dis 2007;**66**:1531–1533. doi: 10.1136/ard.2007.073866

Objectives: A single nucleotide polymorphism (SNP) of the gene encoding protein tyrosine phosphatase type 22 (PTPN22 620W) has recently been described as a strong common genetic risk factor for human autoimmune disease. We have analysed the association of PTPN22 620W in patients with Behçet's disease (BD).

Methods: Genomic DNA was obtained from 270 patients with BD from the UK and the Middle East. Normal controls ($n = 203$) were collected from the same populations. Patients with idiopathic retinal vasculitis from the UK ($n = 136$) were used as disease controls. PTPN22 620W was detected by SSP-PCR analysis and agarose gel electrophoresis.

Results: The results showed an inverse correlation between the presence of PTPN22 620W and Behçet's disease in either patient group tested. There was a greatly reduced prevalence in Middle Eastern compared to UK patients and controls. Finally, there was no association with either UK patients with retinal vasculitis compared with UK controls.

Conclusions: The presence of PTPN22 620W was inversely associated with BD and the distribution of the SNP in the Middle East supports previous findings in the global prevalence.

INTRODUCTION

A functional single nucleotide polymorphism (SNP) of the gene encoding protein tyrosine phosphatase type 22 (PTPN22) (R620W rs 2476601 1858C/T) has recently been described as a strong common genetic risk factor for human autoimmune disease.¹ The PTPN22 gene is located on chromosome 1p13.3 and encodes a lymphoid-specific phosphatase (Lyp) that binds to Csk, an intracellular tyrosine kinase. Csk phosphorylates Lck leading to inhibition of Lck kinase activity. The PTPN22 620W mutation leads to reduced binding of Lyp to Csk, with the overall effect of gain of function mutation, associated with downregulation of early T-cell receptor signalling that results in an increase in autoreactive T cells and autoantibody production, a process thought to be due to defective deletion of autoreactive T cells during thymic maturation or dysfunction of regulatory T cells.² PTPN22 R620W has been associated with rheumatoid arthritis, type 1 diabetes, systemic lupus erythematosus and autoimmune thyroiditis. In contrast, PTPN22 R620W has not been associated with two other presumed autoimmune disorders, multiple sclerosis and Crohn's disease.^{3–4}

Behçet's disease (BD) is a systemic vasculitis characterised by inflammatory lesions of the oro-genital mucosa, eyes, skin, central nervous system and joints. The pathophysiology of BD is unknown but it is characterised by immune hyper-reactivity

mainly affecting elements of the innate immune system. The triggering factor for BD is unclear, although bacterial and viral infections of mucosal surfaces have been implicated.⁵ Idiopathic retinal vasculitis (RV) is a sight-threatening inflammatory disease characterised by breakdown of the blood–retinal barrier, leucocyte infiltration of the retina with photoreceptor cell loss, often leading to blindness. It is usually considered to be an autoimmune, cell mediated, organ specific disease based on the findings of autoreactive T cells and antibodies in patients and its response to immunosuppression.⁶ Both conditions result in intraocular inflammation and both have been associated with HLA and cytokine gene polymorphisms.^{7–8} However, in most cases such polymorphisms are responsible for only a small percentage of the attributable risk. Since both diseases have an autoreactive element in their aetiology, we hypothesised that the PTPN22 R620W mutation might be associated with one or other.

METHODS

After informed consent, blood samples were collected by venepuncture from 270 patients with Behçet's disease, attending the Birmingham and Midland Eye Centre, the Medical Eye Unit at St. Thomas' Hospital, London, the Jordan Hospital, Amman, St John's Hospital, Jerusalem, or University Hospital, Damascus. All patients fulfilled the International Study Group criteria for diagnosis⁹ and underwent a prospective clinical and ophthalmological examination. Normal controls without Behçet's disease were drawn from respective populations ($n = 203$). UK controls were Caucasian transplant donors from which Afro-Caribbean and Far Eastern individuals were removed. Given the high prevalence of BD in the Middle East, local controls were hospital staff and otherwise healthy cataract patients over 50 years of age. New presentation with Behçet's disease after this age is extremely unusual in this population. UK patients with idiopathic retinal vasculitis ($n = 136$) were used as disease controls. This study received the approval of the St Thomas' Hospital and Sandwell Local Ethics Research Committees. DNA was prepared by proteinase K digestion, and salt extraction and stored at -70°C until use. The 620W polymorphism was detected by SSP-PCR using two forward primers and one reverse in two reactions. The separate products of 269 base pairs were run on an agarose gel and read independently by two observers. Associations with disease were sought between allele frequencies. Chi-squared analysis and calculation of odds ratios (ORs) with 95% confidence limits (95% CIs) were carried out using EpiStat.

RESULTS

In this study we have analysed the frequency of PTPN22 R620W to disease in UK and Middle Eastern (ME) patients with Behçet's disease, using UK patients with idiopathic retinal vasculitis as a disease control, and the appropriate control

cohorts. The allelic frequency of the minor T allele was 9.7% in the native UK population, similar to the reported frequency for other populations of European ancestry.³ Overall, the frequency of the PTPN22 R620W mutation was significantly lower in patients with BD when compared to all controls (2.8% v 6.8%; allelic $\chi^2 = 7.7$; $p = 0.005$; OR = 2.4; 95% CI = 1.2 to 4.7) (Table 1). No homozygotes for PTPN22 were identified. However, when separated on geographical origin, PTPN22 R620W frequency remained significantly lower in UK BD patients compared to UK controls (4% v 9.7%; allelic $\chi^2 = 6.0$; $p = 0.001$; OR 2.4; 95% CI = 1.1 to 5.4). By comparison, only 5/140 (1.8%) Middle Eastern BD patients were positive for PTPN22 R620W, and none of the Middle Eastern controls (Fisher's exact test; $p = 0.18$). As the frequency of PTPN22 R620W is so low in individuals of Middle Eastern origin, and a recent study reported that the prevalence of PTPN22 620W in Africans was also very low,¹⁰ such patients ($n = 14$) were removed from our UK cohort of BD patients and the analysis repeated. None of the Middle Eastern or Afro-Caribbean patients were PTPN22 R620W positive, reducing the significance of the association with BD (4.5% v 9.7%; $\chi^2 = 4$; $p = 0.04$; OR 2.1; 95% CI = 1 to 4.7) (Table 1). Finally, there was no difference in the frequency of PTPN22 R620W in patients with idiopathic retinal vasculitis and their healthy UK counterparts (11.9% v 9.7%; allelic $\chi^2 = 0.5$, $p = 0.45$ (NS)) (table 1).

DISCUSSION

This is the first description of the prevalence of PTPN22 R620W being higher in a control population rather than in patients and suggests that for patients with BD in the UK it may provide protection. This is also the first description of PTPN22 R620W expression in patients from the Middle East and, taken together with the very low prevalence in Africans, implies that the polymorphism spread at the same time as migration into western Europe possibly under some novel evolutionary pressure. Interestingly, this does not seem to be the case with migration to the Far East as PTPN22 R620W is not found in Koreans although other polymorphisms are present.¹¹

The functional relevance of our findings has to be confirmed, but our initial hypothesis was that PTPN22 R620W would be associated with BD as it is widely considered to be an autoreactive disease, although it has never been linked to specific autoantibody production. PTPN22 R620W has been associated with susceptibility to Gram positive bacterial infections consistent with a protective role for T cells in the

early immune response to pneumococcal infection.¹⁰ BD has been aetiologically linked to the presence of a variety of Gram positive bacteria resident at mucosal surfaces, in particular *Streptococcus* and *Staphylococcus* species, but the reduced prevalence of PTPN22 R620W in patients with BD compared to the control population in the UK makes it unlikely that impaired responsiveness to bacterial infection is a major factor in the aetiology of the disease. Since it is likely that common susceptibility genes for autoimmunity such as PTPN22 R620W are selected in populations by their additional ability to confer resistance to infectious diseases, recent work on the relationship to *Mycobacterium tuberculosis* infection may be relevant. PTPN22 R620W⁺ individuals were protected against development of overt tuberculosis following immune recognition of *Mycobacteria tuberculosis*. It was suggested that this may have been due to a lack of downregulation of activated T cells and thus a heightened anti-mycobacterial response. Accordingly, the authors proposed that this allele had been selected for by the stronger positive evolutionary pressure of the ability to control TB over-riding the accumulation of a polymorphism inducing selection for autoimmunity.¹² A recent study has also shown a possible link between the acquisition of TB in India and HLA genes. HLA-B*51 was found in significantly more prevalent in patients with TB infection (11/30), whilst HLA-B*52 (0/30) had a protective effect.¹³ Since HLA B*51 is the strongest disease associated gene in populations with Behçet's disease, there may be a link between a reduced prevalence of PTPN22 R620W and an increased prevalence of HLA*B51 as both confer increased susceptibility to TB, an association further supported by the finding of heightened specific immunological responses to mycobacterial heat shock proteins in patients with BD.¹⁴ Since many of these responses are mediated through elements of the innate immune system (NK cells and $\gamma\delta$ T cells) which are upregulated in active Behçet's disease, it would seem reasonable to hypothesise that BD may be a mycobacterially driven auto-inflammatory process as opposed to an autoimmune one.¹⁵ However, it is clear that PTPN22 620W is not the only predisposing factor in BD and that the complex interaction of infectious agents, genetic polymorphism and immune responses has yet to be elucidated.

These results show, firstly, that PTPN22 R620W is inversely associated with BD in the UK population. Secondly, this does not hold for Middle Eastern patients in whom PTPN22 R620W expression does not associate with BD, due to a very low prevalence of the polymorphism in this population.

Table 1 The frequency of the PTPN22 620W mutation in this study

Number of samples	PTPN22 allele number and frequency		p value
	C	T	
BD total ($n = 270$)	525	15 (2.8%)	$\chi^2 = 7.7$, $p = 0.005$
Con total ($n = 234$)	438	30 (6.8%)	OR = 2.4 (1.2 to 4.7)
UK BD total ($n = 130$)	250	10 (4%)	$\chi^2 = 6.0$, $p = 0.001$
UK Con ($n = 169$)	308	30 (9.7%)	OR = 2.4 (1.1 to 5.4)
ME BD ($n = 140$)	275	5 (1.8%)	Fisher's $p = 0.18$
ME Con ($n = 65$)	130	0 (0%)	
UK BD [-AC/ME] ($n = 116$)	222	10 (4.5%)	$\chi^2 = 4.4$, $p = 0.04$
UK Con ($n = 169$)	308	30 (9.7%)	OR = 2.1 (1 to 4.7)
RV patients ($n = 136$)	243	29 (11.9%)	
UK Con ($n = 169$)	308	30 (9.7%)	$\chi^2 = 0.5$, $p = 0.45$ (NS)

UK BD [-AC/ME] denotes the UK cohort with patients of Afro-Caribbean and Middle Eastern origin removed.

Authors' affiliations

Vijay Baranathan, Miles R Stanford, Elizabeth Graham, Departments of Ophthalmology, Guy's, King's and St Thomas' Hospital Medical Schools
Robert W Vaughan, Elli Kondeatis, Clinical Transplantation Laboratory
 Guy's, King's and St Thomas' Hospital Medical Schools
Farida Fortune, Department of Oral Medicine, Queen Mary, University of London
Wafa Madanat, Jordan Hospital, Amman, Jordan
Charlie Kanawati, St. John Eye Hospital, Jerusalem
Marwen Ghabra, University Hospital, Damascus, Syria
Philip I Murray, Graham R Wallace, Academic Unit of Ophthalmology, University of Birmingham, Birmingham

Correspondence to: Dr Graham Wallace, Academic Unit of Ophthalmology, Division of Immunity and Infection, University of Birmingham, Birmingham B15 2TT; g.r.wallace@bham.ac.uk

Accepted 9 July 2007

Published Online First 29 July 2007

REFERENCES

- 1 **Bottini N**, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, *et al.* A functional variant of lymphoid tyrosine phosphatase is associated with type 1 diabetes. *Nat Genet* 2005;**36**:337–8.
- 2 **Vang T**, Congia M, Macis MD, Musumeci L, Orru V, Zavattari P, *et al.* Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant. *Nat Genet* 2005;**37**:1317–9.
- 3 **Gregerson PK**, Lee H-S, Batliwalla F, Begovich AB. PTPN22: setting thresholds for autoimmunity. *Sem Immunol* 2006;**18**:214–23.
- 4 **Bottini N**, Vang T, Cucca F, Mustelin T. Role of PTPN22 in type 1 diabetes and other autoimmune disease. *Sem Immunol* 2006;**18**:207–13.
- 5 **Bonfioli AA**, Orefice F. Behçet's disease. *Semin Ophthalmol* 2004;**20**:199–206.
- 6 Walton RC, Ashmore ED. Retinal vasculitis. *Curr Opin Ophthalmol* 2003;**14**:413–9.
- 7 **Verity DH**, Marr JE, Ohno S, Wallace GR, Stanford MR. Behçet's disease, the silk road and HLA-B51. *Tiss Antig* 1999;**54**:264–72.
- 8 **Chen Y**, Vaughan RW, Kondeatis E, Edelsten CE, Graham EM, Stanford MR, *et al.* Are cytokine gene polymorphisms associated with outcome in patients with retinal vasculitis. *Br J Ophthalmol* 2005;**89**:1013–6.
- 9 **International Study Group for Behçet's Disease**. Criteria for diagnosis of Behçet's disease. *Lancet* 1990;**335**:1078.
- 10 **Chapman SJ**, Khor C, Vannberg FO, Maskell NA, Davies CWH, Hedley EL, *et al.* PTPN22 and invasive bacterial disease. *Nat Genet* 2006;**38**:499–500.
- 11 **Kawasaki E**, Awata T, Ikegami H, Kobayashi T, Maruyama T, Nakanishi K, *et al.* Systematic search for single nucleotide polymorphisms in a lymphoid tyrosine phosphatase gene (PTPN22). *Am J Med Genet A* 2006;**140**:586–93.
- 12 **Gomez LM**, Anaya J-M, Martin J. Genetic influence of PTPN22 R620W polymorphism in tuberculosis. *Hum Immunol* 2005;**66**:1242–7.
- 13 **Lakshmi VV**, Rakh SS, Radhu BA, Priya VHS, Pantula V, Jasti S, *et al.* Role of HLA-B51 and HLA-B52 in susceptibility to tuberculosis. *Infect Gen Evol* 2006;**6**:436–9.
- 14 **Pervin K**, Childerstone A, Shinnick T, Mizushima Y, van der Zee R, Hasan A, *et al.* T cell epitope expression of mycobacterial and homologous human 65 kilodalton heat shock protein peptides in short term cell lines from patients with Behçet's disease. *J Immunol* 1993;**151**:2273–82.
- 15 **Gul, A.** Behçet's disease as an autoinflammatory disorder *Curr Drug Targets Inflamm Allergy* 2005;**4**:81–3.

Keep up to date: sign up for our alerting services

Find out automatically when an article is published on a specific topic or by a particular author. We can also alert you when an article is cited or if an eLetter or correction is published. You can also choose to be alerted when a new issue is published online [and when we post articles Online First]. Check out the New Content Alerts and Citation tracker from the Online tools section on the home page.