European Journal of Human Genetics (2006) 14, 317–321

www.nature.com/ejhg

ARTICLE

Evaluating the role of the 620W allele of protein tyrosine phosphatase PTPN22 in Crohn's disease and multiple sclerosis

Philip L De Jager*[,1,2], Stephen Sawcer[3], Alicja Waliszewska[1,2], Lisa Farwell[2], Gary Wild[4], Albert Cohen[5], Diane Langelier[6], Alain Bitton[4], Alastair Compston[3], David A Hafler[1,2] and John D Rioux[1,2]

[1]*Center for Neurologic Diseases, Department of Neurology, Brigham & Women's Hospital, and Harvard Medical School, Boston, MA, USA;* [2]*Broad Institute, Massachusetts Institute of Technology and Harvard University, Cambridge, MA, USA;* [3]*University of Cambridge Department of Clinical Neurosciences, Addenbrooke's, Hospital, Hills Road, Cambridge, UK;* [4]*Department of Gastroenterology, McGill University Health Centre, McGill University, Montreal, Québec, Canada;* [5]*Montreal Jewish General Hospital, Montreal, Québec, Canada;* [6]*Centre Hospitalier Universitaire de Sherbrooke – Hotel-Dieu, Sherbrooke, Québec, Canada*

The 620W allele of PTPN22 has been associated with susceptibility to several different forms of chronic inflammatory disease, including Type 1 diabetes (T1D), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and autoimmune thyroiditis (AIT). We set out to explore its possible role in two other inflammatory diseases: multiple sclerosis (MS) and Crohn's disease (CD). In our cohort of 496 MS trios from the United Kingdom, we observed reduced transmission of the PTPN22 620W allele. The CD sample consisted of 169 trios as well as 249 cases of CD with their 207 matched control subjects collected in the province of Québec, Canada; there was also no evidence of association between the PTPN22 620W allele and susceptibility for CD. Pooled analyses combining our data with published data assessed a total of 1496 cases of MS and 1019 cases of CD but demonstrated no evidence of association with either disease. Given the modest odds ratios of known risk alleles for inflammatory diseases, these analyses do not exclude a role for the PTPN22 allele in susceptibility to CD or MS, but they do suggest that such a putative role would probably be more modest than that reported so far in T1D, RA, SLE, and AIT.
European Journal of Human Genetics (2006) **14,** 317–321. doi:10.1038/sj.ejhg.5201548; published online 14 December 2005

Keywords: multiple sclerosis; Crohn's disease; PTPN22; association study

Introduction

There has recently been interest in the 620W allele of the hematopoeitic-specific intracellular protein tyrosine phosphatase PTPN22 (OMIM# 600716). This allele is present at a frequency of 8–16% in North American individuals of European descent.[1–4] The functional role of this R620W amino-acid substitution remains unclear at this time; *in vitro*, the presence of the 620W allele decreases the binding affinity of an SH3 domain of the lymphoid-specific phosphatase (LYP) encoded by PTPN22.[1,2] In terms of the function of LYP, RNAi knockdown of PTPN22 results in increased NF-κB activity in the Jurkat T-cell line,[2] and a targeted null allele of PTPN22 in mice is associated with enhanced activation of the T-cell receptor.[5] Given these data on LYP function, several lines of evidence suggest that

***Correspondence: Dr PL De Jager, Center for Neurologic Diseases, Department of Neurology, Brigham & Women's Hospital, Harvard Medical School, 77 Avenue Louis Pasteur, NRB 641, Boston, MA 02115, USA. Tel: +1 617 525 5330; Fax: +1 617 525 5333;**
E-mail: pdejager@rics.bwh.harvard.edu
Received 12 July 2005; revised 14 October 2005; accepted 27 October 2005; published online 14 December 2005

the 620W allele of PTPN22 may lower the threshold of T-cell activation and hence may have a role in susceptibility to inflammatory diseases.[1,2,5]

An association between the 620W allele and susceptibility to disease was first noted in subjects with Type 1 diabetes mellitus (T1D)[1] and prompted us to assess the possible role of the 620W allele in two other inflammatory diseases: Crohn's disease (CD) which affects the gastrointestinal tract and multiple sclerosis (MS) which consists of inflammatory demyelinating episodes in the central nervous system. T cells play an important role in both CD and MS, suggesting a pathophysiological rationale for a possible role of PTPN22 620W in conferring susceptibility to these diseases.[6,7] Since the original report of association with T1D, additional associations have been described with susceptibility to rheumatoid arthritis (RA),[2] systemic lupus erythematosus (SLE),[4] and autoimmune thyroiditis (AIT).[8,9] While it is possible that the 620W allele may be associated with susceptibility to several different inflammatory diseases, an accurate estimate of the extent of the disease risk attributable to this allele awaits further replication efforts in each disease.

Genetic data from some linkage studies also suggested that the area of chromosome 1 in which PTPN22 is found (1p13) is contained in linkage peaks for CD[10] and MS.[11–13] However, 1p has not emerged consistently as a risk locus for either disease. Furthermore, meta-analyses of linkage scans in each disease[14,15] and a recent high-density linkage scan in MS[16] did not provide evidence for a risk locus on 1p. These data suggest that an effect of PTPN22 620W on risk of MS or CD, if it exists, may be modest.

A large sample of North American subjects of European ancestry with MS was recently genotyped for the PTPN22 SNP, and this analysis revealed no statistically significant evidence that 620W was associated with susceptibility to MS.[3] Since then, three other small studies have provided further evidence that the 620W allele has little or no role in susceptibility to MS.[9,17,18] One of these studies analyzed the sample collection of the multiple autoimmune disease genetics consortium (MADGC)[9] which also contains 40 cases of inflammatory bowel disease (a diagnostic category that includes patients with CD as well Ulcerative Colitis) and reported no evidence for association of IBD with PTPN22 620W in this small sample. More recently, 455 CD cases from Canada[19] and 146 CD[20] cases from Germany failed to demonstrate association between PTPN22 620W and CD. Our study extends these assessments and pools all available data to demonstrate that PTPN22 620W does not have a strong effect on susceptibility to either CD or MS.

Materials and methods

Subjects

All affected MS subjects have a diagnosis of MS according to the Poser criteria[21] and are of European descent. All individuals in the UK cohort gave written informed consent for genetic analysis. The study has ethical approval from the Anglia and Oxford Multicentre Ethics Committee. All affected CD subjects fulfill clinical criteria for CD[22] and were consented using a common protocol approved by the institutional review boards of each institution contributing samples in Québec. This collection consists of 169 trios as well as an independent 249 subjects with CD and their 207 matched control subjects. No significant heterogeneity was noted between the allele frequencies in the two CD sample collections or the novel and published MS collections.

Genotytping

All samples were genotyped for rs2476601 using the Sequenom MassArray system as described previously.[23] The genotyping success rate was 97.3% for the MS samples and 93.0% for the CD samples. Genotypes did not deviate from Hardy–Weinberg Equilibrium (HWE; P-value >0.01). there was a Mendelian error (ME) rate of 0.002 in the MS sample and 0 in the CD sample.

Statistical analysis

Association testing Genetic association was assessed by the transmission disequilibrium test (TDT)[24] for collections consisting of trios, as implemented by GENEHUNTER.[25] Association in cases and controls was determined by a standard χ^2 test performed on a 2×2 contingency table.

Combining analyses We computed a pooled estimate of the odds ratio (OR) using the logit method to combine data from different sources, and then derived a confidence interval for the OR as performed for a single 2×2 table, as described previously.[26]

Results

In the 496 MS trios from the UK, the 620W allele frequency of 10% in the UK sample is similar to the reported allele frequency in North American populations of European descent. [1–4,9] TDT analysis reveals that there is no evidence of association of the 620W allele in our MS samples, in fact, there is a nominally significant undertransmission of this allele (Table 1, $P=0.016$). To derive a more accurate assessment of the role of this allele in MS using a family-based approach, we combined our data with the MS trio data collected by Begovich *et al*.[3] The latter 582 North American MS trios of European ancestry from the UCSF collection represent the majority of the cases reported by Begovich *et al*,[3] and all cases fulfill McDonald criteria for MS.[27] The combined collection of 877 MS trios of European ancestry demonstrates no association of MS susceptibility with 620W (OR 0.84, 95% CI 0.66–1.06) (Table 1). To incorporate all extant data on PTPN22 in MS, we also performed a pooled analysis that combines all of the trio data as well as three published case/control studies.[9,17,18]

Table 1 TDT Analysis of MS trio populations and pooled analysis for association with the 1858T allele of PTPN22 which codes for the 620W amino acid allele

MS sample collections	*Subjects with MS*	*T:U (1858T allele)*	*# 1858T (freq) cases*	*# 1858T (freq) controls*	*OR (95% CI)*
Trio studies					
1. UK MS trio	496	63:93			0.677 (0.492–0.933)
2. UCSF MS[3]	381	67:63			1.063 (0.754–1.500)
All trios	877	130:156			0.835 (0.661–1.056)
Case/control studies					
3. MADGC[9]	120		20 (0.083)	351 (0.085)	0.978 (0.611–1.566)
4. Spain MS[17]	120		21 (0.088)	26 (0.065)	1.379 (0.758–2.510)
5. UK MS C/C[18]	379		82 (0.108)	123 (0.103)	1.052 (0.783–1.414)
Pooled analysis					
All studies (1–5)	1496				0.950 (0.806–1.120)

The MS trio collection from the UK genotyped as part of this study is sample collection 1. The analysis of the UCSF MS trio samples (collection 2) used genotype data obtained from Dr J Oksenberg.[3] The frequency of the 1858T allele in the case and control subjects of collections 3–5 was obtained from three published studies.[9,17,18] For example, the MADGC study reported 20T alleles among its 120 subjects with MS and 351T alleles among its 2064 control subjects. The pooled analysis uses the logit method to combine data from all five separate studies, as described in the Materials and methods section; as a result, healthy control subjects from collections 1 and 2 are not used when each of the three case/control logit values (collections 3–5) are calculated. The frequency of the 1858T allele in the parents of the UK trio sample (1) is 0.098 and the frequency of the parents of the UCSF trio sample (2) is 0.101.

Table 2 Pooled analysis of CD samples for association with the 1858T allele of PTPN22 which codes for the 620W amino acid allele

CD sample collections	*Affected Subjects*	*T:U*	*# 1858T (freq) cases*	*# 1858T (freq) controls*	*OR (95% CI)*
Newly genotyped collections from Québec, Canada					
1.Trios[a]	169	21:18			1.167 (0.622–2.188)
2.Case/control	249		30 (0.048)	16 (0.040)	1.194 (0.642–2.219)
All new cases (1&2)	418				1.180 (0.759–1.836)
Published reports					
3.Canadian C/C[19]	455		68 (0.075)	25 (0.067)	1.147 (0.713–1.843)
4.German C/C[20]	146		25 (0.086)	53 (0.104)	0.804 (0.488–1.323)
Pooled analysis[b]					
All studies (1–4)	1019				1.044 (0.796–1.369)

[a]The frequency of the 1858T allele in the parents of sample population (1) (trios from Québec) is 0.083.
[b]The samples from the MADGC collections (40 cases of IBD)[9] are not added to the pooled analysis given the limited number of individuals with CD. The allele frequencies for sample collections (3) and (4) were obtained from published data.[19,20]

This analysis also failed to demonstrate association of MS susceptibility with PTPN22 620W (OR 0.95, 95% CI 0.81–1.12) (Table 1). Finally, a recent report suggests that the OR for an association of 620W with RA susceptibility in a large Swedish cohort is much greater in males (1.76) than females (1.19) (R. Plenge, personal communication). We therefore stratified the pooled trio data by gender but saw no significant evidence of association with MS susceptibility with either gender (data not shown).

To examine subjects with CD, we genotyped 169 trios as well as 249 cases and 207 control subjects collected in the province of Québec, Canada. The minor allele frequency of 0.08 for 620W in the CD trio samples is similar to those described in other populations of European descent,[1–4,9,17,18] but the frequency of 0.04 in the case/control sample is less than expected. The combined TDT and case/control analysis reveals no evidence of association of the 620W allele of PTPN22 with susceptibility for CD in our subject sample (OR 1.18, 95% CI 0.76–1.84) (Table 2). A pooled analysis (Table 2) that combines our data with those of the other significant studies of PTPN22 in CD[19,20] similarly demonstrates no evidence of association between PTPN22 620W and susceptibility to CD (OR 1.04, 95% CI 0.80–1.37). An analysis stratified by gender also did not reveal a significant association (data not shown). This pooled analysis of CD collections is substantial, but, given the modest OR's associated with the PTPN22 620W allele in other diseases, this analysis should not be considered as definitive in the evaluation of the role of PTPN22 in CD.

Discussion

Reported estimates of the OR for 620W association with disease vary significantly from one study and one disease to another; however, an OR of 1.3 has clearly been observed for this allele in certain SLE and RA studies[4] (Plenge, personal communication). Taking the OR as a rough estimate of GRR, we therefore cannot rule out that the 620W allele is associated with susceptibility to MS or CD. With the pooled MS sample of 1496 affected subjects, we have an estimated 89% power to exclude an OR >1.3 but only 59% power to exclude an OR >1.2 (assuming a multiplicative model of disease risk, allele frequency of 0.1, and an α of 0.05)(Genetic Power Calculator, http://statgen.iop.kcl.ac.uk/gpc/).[28] Thus, it appears that the PTPN22 620W allele does not have a strong effect on risk of MS. It is intriguing that the 496 trios from the UK genotyped as part of this study demonstrate a nominally significant overtransmission of the major R620 allele. Given the lack of supporting evidence for this observations in the other studies[3,9,17,18] and the pooled analysis, this observation is likely to represent a statistical fluctuation. However, since a detailed haplotype-based analysis of the PTPN22 gene has not yet been performed in subjects with MS, we cannot rule out the possibility of allelic heterogeneity. An MS risk allele could exist and be in linkage disequilibrium with the PTPN22 R620 allele.

The CD sample is smaller than the MS sample and has a somewhat lower frequency of the 620W allele; therefore, we cannot exclude the possibility that PTPN22 has a modest effect on susceptibility to CD. With the pooled analysis of 1019 cases of CD, we have an estimated 56% power to exclude an OR of 1.3 and 92% power to exclude an OR of 1.5 for the PTPN22 620W allele (assuming a multiplicative model of disease risk, allele frequency of 0.06, and an α of 0.05)(Genetic Power Calculator, http://statgen.iop.kcl.ac.uk/gpc/).[28] Thus, while evidence mounts that the 620W allele of PTPN22 may have an effect on susceptibility to T1D, RA, AIT, and SLE, the evidence so far points to a lack of association between this allele and susceptibility for either MS or CD. However, additional subjects from other collections will be needed to definitively exclude a role for the 620W allele of PTPN22 in these diseases.

Acknowledgements

We thank Drs AB Begovich and J Oksenberg for sharing their genotyping data. PLD is the William C Fowler scholar in Multiple Sclerosis and is supported by an NINDS K08 grant as well as the Clinical Investigator Training Program: Harvard-MIT Health Sciences and Technology Beth Israel Deaconess Medical Center, in collaboration with Pfizer Inc. JDR is supported by grants from the NIDDK and the CCFA. The authors have no conflicts of interest to report.

References

1 Bottini N, Musumeci L, Alonso A *et al*: A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat Genet* 2004; **36**: 337–338.
2 Begovich AB, Carlton VE, Honigberg LA *et al*: A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am J Hum Genet* 2004; **75**: 330–337.
3 Begovich AB, Caillier SJ, Alexander HC *et al*: The R620W polymorphism of the protein tyrosine phosphatase PTPN22 is not associated with multiple sclerosis. *Am J Hum Genet* 2005; **76**: 184–187.
4 Kyogoku C, Langefeld CD, Ortmann WA *et al*: Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. *Am J Hum Genet* 2004; **75**: 504–507.
5 Hasegawa K, Martin F, Huang G *et al*: PEST domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory T cells. *Science* 2004; **303**: 685–689.
6 Wen Z, Fiocchi C: Inflammatory bowel disease: autoimmune or immune-mediated pathogenesis? *Clin Dev Immunol* 2004; **11**: 195–204.
7 Hafler DA, Slavik JM, Anderson DE *et al*: Multiple sclerosis. *Immunol Rev* 2005; **204**: 208–231.
8 Smyth D, Cooper JD, Collins JE *et al*: Replication of an association between the lymphoid tyrosine phosphatase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. *Diabetes* 2004; **53**: 3020–3023.
9 Criswell LA, Pfeiffer KA, Lum RF *et al*: Analysis of families in the multiple autoimmune disease genetics consortium (MADGC) collection: the PTPN22 620W allele associates with multiple autoimmune phenotypes. *Am J Hum Genet* 2005; **76**: 561–571.
10 Cho JH, Nicolae DL, Gold LH *et al*: Identification of novel susceptibility loci for inflammatory bowel disease on chromosomes 1p, 3q, and 4q: evidence for epistasis between 1p and IBD1. *Proc Natl Acad Sci USA* 1998; **95**: 7502–7507.
11 Hensiek AE, Roxburgh R, Smilie B *et al*: Updated results of the United Kingdom linkage-based genome screen in multiple sclerosis. *J Neuroimmunol* 2003; **143**: 25–30.
12 Dyment DA, Sadovnick AD, Willer CJ *et al*: An extended genome scan in 442 Canadian multiple sclerosis-affected sibships: a report from the Canadian Collaborative Study Group. *Hum Mol Genet* 2004; **13**: 1005–1015.
13 Kenealy SJ, Babron MC, Bradford Y *et al*: A second-generation genomic screen for multiple sclerosis. *Am J Hum Genet* 2004; **75**: 1070–1078.
14 van Heel DA, Fisher SA, Kirby A *et al*: Genome scan meta-analysis group of the IBD international genetics consortium. Inflammatory bowel disease susceptibility loci defined by genome scan meta-analysis of 1952 affected relative pairs. *Hum Mol Genet* 2004; **13**: 763–770.
15 GAMES; Transatlantic Multiple Sclerosis Genetics Cooperative: A meta-analysis of whole genome linkage screens in multiple sclerosis. *J Neuroimmunol* 2003; **143**: 39–46.
16 Sawcer S, Ban M, Maranian M *et al*: A high-density screen for linkage in multiple sclerosis. *Am J Hum Genet* 2005; **77**: 454–467.
17 Matesanz F, Rueda B, Orozco G *et al*: Protein tyrosine phosphatase gene (PTPN22) polymorphism in multiple sclerosis. *J Neurol* 2005; **252**: 994–995.
18 Hinks A, Barton A, John S *et al*: Association between the PTPN22 gene and rheumatoid arthritis and juvenile idiopathic arthritis in a UK population: further support that PTPN22 is an autoimmunity gene. *Arthritis Rheum* 2005; **52**: 1694–1699.
19 van Oene M, Wintle RF, Liu X *et al*: Association of the lymphoid tyrosine phosphatase R620W variant with rheumatoid arthritis, but not Crohn's disease, in Canadian populations. *Arthritis Rheum* 2005; **52**: 1993–1998.
20 Wagenleiter SE, Klein W, Griga T *et al*: A case-control study of tyrosine phosphatase (PTPN22) confirms the lack of association with Crohn's disease. *Int J Immunogenet* 2005; **32**: 323–324.

21 Poser CM, Paty DW, Scheinberg L *et al*: New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol* 1983; **13**: 227–231.
22 Lennard-Jones JE: Classification of inflammatory bowel disease. *Scand J Gastroenterol* 1989; **170**: 2–6.
23 Vermeire S, Wild G, Kocher K *et al*: CARD15 genetic variation in a Quebec population: prevalence, genotype-phenotype relationship, and haplotype structure. *Am J Hum Genet* 2002; **71**: 74–83.
24 Spielman RS, Ewens WJ: The TDT and other family-based tests for linkage disequilibrium and association. *Am J Hum Genet* 199; **59**: 983–989.
25 Daly MJ, Kruglyak L, Pratt S *et al*: GENEHUNTER 2.0-a complete linkage analysis system. *Am J Hum Genet* 1998; **63**: S286.
26 Morris JA, Gardner MJ: Calculating confidence intervals for relative risks (odds ratios) and standardised ratios and rates. *Br Med J (Clin Res Ed)* 1988; **296**: 1313–1316.
27 McDonald WI, Compston A, Edan G *et al*: Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001; **50**: 121–127.
28 Purcell S, Cherny SS, Sham PC: Genetic power calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 2003; **19**: 149–150.