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Genetic Influence of *PTPN22* R620W Polymorphism in Tuberculosis

Luis M. Gomez, Juan-Manuel Anaya, and Javier Martin

ABSTRACT: The *PTPN22* gene codes for an intracellular lymphoid-specific phosphatase (Lyp) that has a negative regulatory effect on T-cell activation. Because Lyp is an important molecule involved in the inflammatory response, and its levels are increased in cells that participate in the immune response against *Mycobacterium tuberculosis*, we hypothesized that the functional *PTPN22* C1858T polymorphism could be a genetic factor predisposing to the development of tuberculosis (TB). Accordingly, we undertook an association study in which 113 patients with pulmonary TB and 161 matched healthy controls stratified by the tuberculin skin test (TST) were examined. Significant skewing was observed when T allele frequencies of patients with TB and all controls were compared ($P = 0.04$, odds ratio = 0.3; 95% confidence interval = 0.08–1.04) and frequencies of patients with TB and TST+ healthy controls were compared ($P = 0.01$, odds ratio = 0.2; 95% confidence interval = 0.05–0.79). No stratification was detected between patients and control samples. These results suggest that the T allele may be a factor protecting against development of TB once the immune system recognizes *M. tuberculosis* (*i.e.,* TST+ individuals), whereas the C allele may be a risk factor for development of overt TB. The results also indicate that an association opposite that between the *PTPN22* polymorphism and TB exists between TB and autoimmunity. *Human Immunology 66, 1242–1247 (2005).* © American Society for Histocompatibility and Immunogenetics, 2006. Published by Elsevier Inc.

KEYWORDS: *PTPN22*; tuberculosis; tuberculin skin test; delayed-type hypersensitivity; autoimmunity

ABBREVIATIONS

Csk	C-terminal Src tyrosine kinase
CI	confidence interval
DC	dendritic cell
DTH	delayed-type hypersensitivity
Grb2	growth factor receptor-bound protein 2
NK	natural killer
OR	odds ratio
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PTPN22	protein tyrosine phosphatase nonreceptor 22
SLE	systemic lupus erythematosus
SNP	single-nucleotide polymorphism
T1D	type 1 diabetes
TB	tuberculosis
TST	tuberculin skin test

INTRODUCTION

Tuberculosis (TB) remains one of the most common causes of illness and mortality in humans, and it is estimated that approximately one-third of the world's population has been infected with *Mycobacterium tuberculosis* [1]. The natural history of TB follows a variable course after initial infection, with only 10% of those infected developing clinical disease. Although associated illnesses, medical treatments, and malnutrition may increase the risk of developing disease, these conditions, except for human immunodeficiency virus (HIV) infection, account for only a small proportion of TB cases today [1].

In the majority of infected individuals, active natural and acquired immune responses successfully control *M. tuberculosis.* Natural immune mechanisms such as dendritic cells (DCs), natural killer (NK) cells, macrophages, and neutrophils probably have an important role in the primary response to *M. tuberculosis* and may suffice to control infection in some individuals. In the majority of individuals, however, acquired immune responses are necessary for the control of *M. tuberculosis* infection [2].

The *PTPN22* (protein tyrosine phosphatase nonreceptor 22) gene maps to chromosome 1p13.3-p13.1 [3] and encodes an intracellular lymphoid-specific phosphatase

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known as Lyp, which contains an N-terminal catalytic domain and a noncatalytic C terminus with four proline-rich domains [3]. Lyp dephosphorylates the kinases Lck, Fyn, and Zap-70, all known to be important in T-cell signaling. An additional function of Lyp is to downregulate activation of T cells by binding to C-terminal Src tyrosine kinase (Csk). Csk is an essential suppressor of kinases that mediates T-cell activation [3]. In addition, Lyp binds to the adaptor molecule Grb2 (growth factor receptor-bound protein 2), and this interaction is thought to play a negative regulatory function in T-cell signaling [4]. It is expressed primarily in hematopoietic tissues [3, 4], such as thymus, spleen, bone marrow, and peripheral blood mononuclear cells (PBMCs). Recent findings confirmed and extended these results in specific PBMC subsets as well as in NK cells, neutrophils, and CD8+ cells [5], all of which can be detected in the tuberculous granuloma [6].

Recent findings have demonstrated that a single-nucleotide polymorphism (SNP) of *PTPN22* (C1858T; dsSNP no, rs2476601, R620W) placed at the P1 motif does not bind Csk, preventing formation of the complex and, therefore, suppression of T-cell activation [7]. In Caucasians, the *PTPN22* T allele is a risk factor for some autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, autoimmune thyroid diseases, and type 1 diabetes (T1D) [5, 7–10], but not for others, such as multiple sclerosis [9, 11]. In Colombians, similar results have been reported for SLE, primary Sjögren's syndrome, and T1D [12].

Because Lyp is an important molecule involved in the immune and inflammatory response and its levels are increased in cells that participate in the immune response against *M. tuberculosis*[5, 6], we hypothesized that the functional *PTPN22* C1858T polymorphism could be an important genetic factor predisposing to the development of TB.

MATERIALS AND METHODS

Patients

One hundred and thirteen patients (96 women, 17 men) with pulmonary TB were enrolled in the study at the time of treatment for their disease. Their mean age was 40 ± 15. TB was diagnosed by detection of alcohol-resistant acid-fast bacilli in sputum or by isolation of *M. tuberculosis* in culture. In all cases, patients with TB were negative for HIV 1/2 infection (by Axsym Assays, Abbott Laboratories, Chicago, IL, USA). Patients had been enrolled at the Corporación para Investigaciones Biológicas (CIB), Medellin, Colombia, as part of a project on the immunogenetics of TB [13]. Three patients with TB had a previous history of autoimmune hemolytic anemia, discoid lupus erythematosus, or systemic lupus erythematosus.

As a control population, we included 161 individuals (112 women, 49 men) older than 18 and from both sexes, without inflammatory, autoimmune disease or a history of chronic infectious disease, including TB and HIV infection. They were matched to patients with respect to gender, ethnicity, and socioeconomic status and were unrelated to the patients.

Historical and genetic evidence suggests that the recently established population of Antioquia is potentially useful for genetic mapping. This population was established in the 16th to 17th centuries and flourished in relative isolation until the late 19th century [14]. The admixture between Paisa and African or Amerindian populations has been historically documented as low [15]. The identity coefficient method has estimated the ancestral ethnic components as 85% Caucasian and 15% Amerindian [16]. The African contribution to the Paisa community was estimated as being not significantly different from zero [17].

This research was conducted in compliance with Resolution 008430 of 1993 of the Ministry of Health of Colombia, and was classified as research with minimal risk. The local ethics committee approved the study.

Tuberculin Skin Test

All control individuals underwent testing to assess the delayed-type hypersensitivity (DTH) skin test response to 0.1 ml (5 TU) of the purified protein derivative (PPD) antigen (Tuberculin PPD Powder, Master Lot No. 154616, from the Public Health Service, Centers for Disease Control and Prevention, Atlanta, GA, USA) injected intradermally into the forearm, following the Mantoux method [18]. The skin test response was measured as the diameter of induration 48–72 hours after injection. We classified these subjects into those who were naturally infected with *M. tuberculosis* (*i.e.,* diameter of induration ≥10 mm) and those who were uninfected at the time of DTH ascertainment (diameter of induration < 10 mm); 75 (~47%) subjects were considered to be naturally infected, and 86 (~53%) were considered uninfected, that is, tuberculin skin test-negative (TST-). The mean age of these individuals was 45 ± 16 for TST- healthy controls and 47 ± 15 for TST+ healthy controls. All of them were negative for TB disease.

Genotyping

Genomic DNA was isolated from 10-ml EDTA-anticoagulated blood samples using the standard salting-out technique. Genotyping for *PTPN22* C1858T was carried out using a Custom TaqMan SNP Genotyping Assays method (Applied Biosystems, Foster City, CA, USA). The primer sequences were 5′-CCAGCTTC-

TABLE 1 Frequency of *PTPN22* C1858T genotypes and alleles with respect to TST status

	TB patients	All controls	TST+ controls
PTPN22 C1858T genotype	(n = 113)	(n = 161)	(n = 75)
CC	110 (97.3)[a]	147 (91.3)	66 (88)
CT	3 (2.7)	14 (8.7)[b]	9 (12)[c]
TT	0 (0)	0 (0)	0 (0)
PTPN22 C1858T allele	(n = 226)	(n = 322)	(n = 150)
C	223 (98.7)	308 (95.7)	141 (94)
T	3 (1.3)	14 (4.3)[d]	9 (6)[e]

[a] Number (%).

[b] Comparison between patients and all controls for genotype CT versus genotype CC: OR = 0.28, 95% CI = 0.08–1.02, p= 0.04.

[c] Comparison between patients and TST+ controls for genotype CT versus genotype CC: OR = 0.2, 95% CI = 0.05–0.76, p= 0.01.

[d] Comparison between patients and all controls for allele T versus allele C: OR = 0.3, 95% CI = 0.08–1.04, p= 0.04.

[e] Comparison between patients and TST+ controls for allele T versus allele C: OR = 0.2, 95% CI = 0.05–0.79, p= 0.01.

CTCAACCACAATAAATG-3'(sense) and 5'-CAACTGCTCCAAGGATAGATGATGA-3' (antisense). The Taqman minor groove binder probe sequences were 5'-caggtgtccatacagg-3', and 5'-caggtgtccgtacagg-3′. The probes were labeled with the fluorescent dyes VIC and FAM, respectively. Polymerase chain reaction (PCR) was carried out in total volume of 12.5 μl using the following amplification protocol: denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 92°C for 15 seconds, and annealing and extension at 60°C for 1 minute. After PCR, each sample genotype was determined by measuring allele-specific fluorescence in the ABI Prism 7000 Sequence Detection System, using SDS 1.1 software for allele discrimination (Applied Biosystems). To confirm the genotype obtained by the Taqman 5'-allele discrimination assay, PCR restriction fragment length polymorphism was performed on representative samples from each genotype as previously described [7]. Briefly, we used the forward primer 5′-TCACCAGCTTCCTCAACCACA-3′ and the reverse primer 5′-GATAATGTTGCTTCAACGGAATTTA-3′. The genotypes were identified by *Xcm*I restriction endonuclease digestion, which recognizes its target sequence only when the *PTPN22* 1858T allele is present. Digestion products were resolved on 3% agarose gels.

Statistical and Genetic Analysis

Allele and genotype frequencies of *PTPN22* C1858T were obtained by direct counting. Data were managed using the GraphPad InStat V3.00 for Windows (GraphPad Software, San Diego, CA, USA) and Genepop v3.4 software *(*http://wbiomed.curtin.edu.au/genepop*)*. Differences between allele and genotype frequencies were determined using the χ^2 test and the two-sided Fisher exact test as appropriate. Crude odds ratios (ORs) were calculated with 95% confidence intervals (CIs). A p value <0.05 was considered statistically significant. Population heterogeneity among the case and control sets was examined using Wright's F statistic according to the nonbiased method of Weir and Cockerham [19].

RESULTS

We examined the *PTPN22* C1858T SNP in 113 patients with TB and in 161 healthy controls stratified by TST. Genotypic and allelic frequencies corresponding to *PTPN22* C1858T SNP among patients with TB and controls are listed in Table 1. Both patients and controls were in Hardy–Weinberg equilibrium. There was no stratification between patients and control samples, because the F_{st} subdivision coefficient was not significantly different from zero (Table 2). Thus, patients and controls had a similar genetic background.

When genotypes in a 2 × 2 contingency table were compared among patients with TB and all controls, an association with protection was observed in T allele-carrying genotypes (C/T genotype vs C/C genotypes, P = 0.04, OR = 0.28, 95% CI = 0.08–1.02). This statistically significant difference was increased when T allele-carrying genotypes of patients with TB were compared with TST+ healthy controls (P = 0.01, OR = 0.2, 95% CI = 0.05–0.76).

This statistically significant skewing was also observed when T allele frequencies were compared between TB patients and all controls (P = 0.04, OR = 0.3, 95% CI = 0.08–1.04) and between TB patients and TST+ healthy controls (P = 0.01, OR = 0.2, 95% CI = 0.05–0.79). We could not detect individuals carrying the TT genotype among patients and controls. In addition, the frequency of the T allele observed in our Colombian sample set was very low (4.3%) compared with that observed in some Caucasian populations (≥7%) [9, 10].

TABLE 2 Estimates of population subdivision[a]

	f	F	θ
Locus[b]	−0.0283	0.0070	−0.0355
Replicas	10000	10000	10000
95% CI	95.0	95.0	95.0

[a] θ, f, and F estimates represent the nonbiased estimates of Wright's classic F_{is}, F_{it}, and F_{st} subdivision coefficients; 95% CI = confidence interval of 95% of the estimates after the bootstrap process of 10,000 replicas. As can be seen, there was no subdivision at the individual–subpopulation, individual–total population (patients and controls together), and subpopulation–total population levels.

[b] Corresponds to *PTPN22* locus.

We also examined the influence of *PTPN22* C1858T with respect to gender. There were no significant differences between *PTPN22* C1858T alleles and genotypes between men and women (data not shown).

DISCUSSION

Many studies, notably those on monozygotic and dizygotic twins, indicate that inherited genetic factors play a key role in determining susceptibility and resistance to overt TB following infection [20–23]. The immune interactions between the host and the highly complex molecular structure of *M. tuberculosis* are multifactorial. From the standpoint of the host, resistance to infection depends on the ability of macrophages to phagocytose and destroy the bacilli; and this, in turn, is affected by class II HLA-determined antigen presentation, the activation of macrophages by T cell-derived cytokines and vitamin D [24], and granuloma formation. Several of the genes involved in these processes have been identified, notably the HLA-DR and HLA-DQB1 loci, which determine which mycobacterial antigens are presented to helper T cells [25, 26]. Our results indicate that in addition to other non-HLA genes, such as *NRAMP1,* vitamin D receptor gene, and *TNF* [13, 27, 28],*PTPN22* also influences susceptibility/resistance to the development of pulmonary TB.

The *PTPN22* gene product (*i.e.,* Lyp) is an important downregulator of T-cell activation and other cell subsets such as NK cells and neutrophils, in part through its physical interaction with Csk and probably through its interaction with the adaptor molecule Grb2 [4, 29]. Several reports have demonstrated that the T allele is a risk factor for some but not all autoimmune diseases [5, 7–12]. On the basis of animal models, *PTPN22* may play a role in diseases characterized by lymphoproliferation. *PTPN22* knockout mice display rather subtle changes in a number of immune parameters, such as enlargement of the spleen and lymph nodes, that are accompanied by spontaneous formation of germinal centers and higher levels of antibodies [30]. Nevertheless, the majority of data on the role of this protein in T-cell activation stems from work on the mouse ortholog, PEP, which is only 70% homologous to Lyp [30].

To our knowledge, a role for the *PTPN22* C1858T SNP in the susceptibility to acquire infectious diseases, such as TB, has not been reported. We believe that this functional SNP could influence the susceptibility to develop TB, because Lyp is an important molecule involved in the inflammatory response, and its levels are augmented in cells that participate in the immune response against *M. tuberculosis* [5]. Our results have shown that T allele carriers are protected against disease once the immune system recognizes *M. tuberculosis* (*i.e.,* TST+ individuals), whereas C allele carriers are prone to development of overt TB.

The question that arises is how to translate this genetic result into physiopathology. Because this SNP is functionally important and *PTPN22* T allele carriers do not have the downregulatory effect of Lyp on T-cell responses, these individuals could respond better to *M. tuberculosis* and control the infection. In those individuals who are *PTPN22* T allele carriers and are TST-, other mechanisms may play an additional role in resistance to mycobacteria, or these persons may have never been in contact with the infectious agent. Because not a single TST- individual was immunosuppressed or had a chronic disease, anergy is not a plausible explanation for their DTH status.

Increased accumulation of neutrophils in the granuloma and increased chemotaxis are two phenomena observed in TB [31]. At the site of multiplication of bacilli, neutrophils are the first cells to arrive, followed by NK cells, γ/δ T cells, and α/β T cells [2]. Studies in humans have demonstrated that neutrophils provide agents such as defensins, which are lacking in macrophage-mediated killing [32]. NK cells are also the effector cells of innate immunity. These cells may directly lyse pathogens or can lyse infected monocytes. *In vitro* co-culture with live *M. tuberculosis* results in the expansion of NK cells, implying that they may be important responders to *M. tuberculosis* infection *in vivo* [33, 34]. During early infection, NK cells are capable of activating phagocytic cells at the site of infection [34]. However, because the influence of the *PTPN22* C1858T polymorphism on protein production may differ with tissue and cell type [5], further studies are needed to assess the relative influence of the *PTPN22* C1858T polymorphism in the local expression of Lyp in the lung and in the tuberculous granuloma.

Noteworthy, the association of the *PTPN22* polymorphism with disease in our population is opposite that between TB and autoimmunity [12], suggesting positive selection. Natural selection for resistance to a pathogen can lead to decrease in the frequency of alleles that are otherwise deleterious [35]. If protection from infection is a stronger selective force than negatively selected phenotype, the deleterious allele will accumulate in the population as long as the infectious agent remains prevalent. Thus, infectious diseases such as TB may exert immunological protection against autoimmunity (by homeostatic competition, bystander suppression, or toll-like receptor stimulation) [36, 37], favoring at the same time a selective genetic pressure that would increase the risk of autoimmune diseases [13, 35].

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