Brief Genetics Report

Genetic Association Between a Lymphoid Tyrosine Phosphatase (*PTPN22*) and Type 1 Diabetes

Weipeng Zheng and Jin-Xiong She

The lymphoid-specific phosphatase (LYP) encoded by *PTPN22* is involved in preventing spontaneous T-cell activation by dephosphorylating and inactivating T-cell receptor–associated Csk kinase. We have genotyped 396 type 1 diabetic patients and 1,178 control subjects of Caucasian descent from north central Florida and report a strong association between type 1 diabetes and a polymorphism (R620W) in the *PTPN22* gene. The homozygous genotype for the T allele encoding the 620W residue is associated with an increased risk for developing type 1 diabetes (odds ratio [OR] = 3.4, $P < 0.008$), and the heterozygous genotype C/T had an OR of 1.7 ($P = 6 \times 10^{-6}$). The C/C homozygous genotype is protective against type 1 diabetes (OR = 0.5, $P = 6 \times 10^{-6}$). Furthermore, transmission disequilibrium analysis of 410 affected sibpair and simplex families of Caucasian descent indicated that the type 1 diabetes–associated T allele is transmitted more often (57.2%) than randomly expected ($P < 0.003$). Together with previous reports of the association between *PTPN22* and type 1 diabetes, as well as rheumatoid arthritis and systemic lupus erythematosus, these results provide compelling evidence that LYP is a critical player in multiple autoimmune disorders. *Diabetes* 54:906–908, 2005

Two major strategies have been used to identify human susceptibility genes for type 1 diabetes and other complex diseases. The first strategy involves a genome scan for linkage using affected sibpair families and polymorphic markers throughout the entire human genome. This strategy has resulted in the identification of >20 large genomic regions that may contain type 1 diabetes genes (1–3). However, thousands or tens of thousands of families are required to detect genes with weak effect or to narrow disease genes to a reasonably small interval suitable for positional cloning (4). The second strategy for disease gene discovery involves testing the association between genetic markers and the disease in affected families or case/control subjects, an approach that is required for the identification of complex disease genes and has been successfully used for the discovery of type 1 diabetes susceptibility genes (5–8). However, genetic markers in strong linkage disequilibrium with the disease-causing (etiological) polymorphism must be used for the successful detection of association. Conventionally, association studies are applied to specific candidate genes of presumed importance in the disease. We have used this approach to evaluate association between type 1 diabetes and the lymphoid-specific phosphatase (LYP) encoded by *PTPN22* (9). LYP belongs to a family of protein tyrosine phosphatases (PTPs) that are involved in preventing spontaneous T-cell activation by dephosphorylating and inactivating T-cell receptor–associated kinases and their substrates (10,11). *PTPN22* is specifically expressed in lymphocytes (9,12) and associated with the SH3 domain of the Csk kinase, which suppresses the mediators of T-cell receptor signaling, i.e., the Src family kinases (Lck and Fyn) (11,13). LYP is one of the most powerful inhibitors of T-cell activation. Recently, a missense mutation in the *PTPN22* gene was found to be associated with multiple autoimmune diseases including type 1 diabetes (14), rheumatoid arthritis (12), and systemic lupus erythematosus (15). It has also been shown that the mutation decreases the binding affinity of LYP to CSK, suggesting a functional relevance to T-cell activation. However, these studies used a case/control study design to demonstrate disease association, and there is still a possibility that the observed associations were due to a mismatch of ethnicity between patients and control subjects. In this report, we confirm the association between type 1 diabetes and *PTPN22* in a case/control dataset as well as in a large collection of diabetic families.

RESEARCH DESIGN AND AND METHODS

Genotyping. A 218-bp fragment containing the C1858T single nucleotide polymorphism (R620W) within the coding region of the *PTPN22* gene was amplified using the forward primer PTPN22_rs247_F1 (5′ACTGATAATGTT GCTTCAACGG) and the reverse primer PTPN22_RS247_R1 (5′TCACCAGCT TCCTCAACCAC). The amplification reaction contains 2 μl (20 ng) genomic DNA, 1.2 μl 10× PCR buffer, 1.2 μl dNTP (1.25 mmol/l), 0.3 μl of each primer (20 pmol/μl), 0.6 μl DMSO, and 0.1 μl *Taq* polymerase in a 12-μl reaction. Samples were initially denatured for 2 min at 94°C followed by 35 cycles of 94°C for 30 s, 30 s at 60°C, and 30 s at 72°C and an additional extension of 2 min at 72°C. Amplified products (12 μl) were digested using 10 units of *Rsa*I (New England Biolabs) per reaction overnight at 37°C. Digested products were electrophoresed on a 2.5% agarose gel. DNA fragments were visualized by ethidium bromide. The mutant 1858T allele cannot be digested and yields

From the Center for Biotechnology and Genomic Medicine, Medical College of Georgia, Augusta, Georgia.

Address correspondence and reprint requests to Dr. Jin-Xiong She, Medical College of Georgia, Center for Biotechnology and Genomic Medicine, 1120 15th St., PV6B108, Augusta, GA 30912. E-mail: jshe@mail.mcg.edu.

Received for publication 19 August 2004 and accepted in revised form 1 December 2004.

LYP, lymphoid-specific phosphatase; PTP, protein tyrosine phosphatase.

TABLE 1
Association between *PTPN22* and type 1 diabetes

	C/C	C/T	T/T	C/T + T/T
Patients (n = 396)	290 (73.2)	97 (24.5)	9 (2.3)	106 (26.8)
Control subjects (n = 1,178)	984 (83.5)	186 (15.8)	8 (0.7)	194 (16.5)
OR (95% CI)	0.5 (0.4–0.7)	1.7 (1.3–2.3)	3.4 (1.3–8.9)	1.9 (1.4–2.4)
P	6×10^{-6}	9×10^{-5}	0.008	6×10^{-6}

Data are n (%) or OR (95% CI).

one fragment of 218 bp, while the 1858C allele yields two fragments of 176 bp and 46 bp.

Genomic DNA was obtained from members of 410 sibpair families with type 1 diabetes. The collection includes U.S. Caucasian families from the southeast as well as families in the HBDI (Human Biological Data Interchange). The sporadic case and control subjects were obtained from north central Florida. All diabetic patients used in this study were diagnosed using the criteria of the National Diabetes Data Group. The control subjects are normal subjects without a family history for type 1 diabetes. The clinical information on the patients has been described in previous publications (8,16,17).

Statistical analysis. The transmission disequilibrium test was used to assess association. A χ^2 test was used to evaluate the deviation from 50% expectation of transmission from heterozygous parents to affected siblings. Case/control association was assessed by the 2 × 2 contingency table χ^2 test.

RESULTS AND DISCUSSION

The C1858T single nucleotide polymorphism within the PTPN22 gene was genotyped for 396 Caucasian patients with type 1 diabetes and 1,178 matched control subjects obtained from north central Florida. The genotypic frequencies are presented in Table 1. The homozygous mutant genotype (T/T) is uncommon in the control population (0.7%) but has an increased frequency in type 1 diabetic patients (2.3%). This genotype confers an odds ratio (OR) of 3.4 (P = 0.008) (Table 1). The heterozygous genotype C/T is found in 15.8% of the control subjects and 24.5% of the patients and confers an OR of 1.7 ($P = 9 \times 10^{-5}$). The wild-type homozygous genotype (C/C) had a higher frequency in the control subjects (83.5%) compared with type 1 diabetic patients (73.2%) (OR = 0.5, $P = 6 \times 10^{-6}$). We also analyzed 103 patients and 302 control subjects of Chinese origin, but the mutant allele was not found in the Chinese population.

Although case/control associations in this study, as well as in another recent report (15), provided strong evidence for the association between type 1 diabetes and *PTPN22*, it is still possible that the associations were due to mismatch of ethnicity. Therefore, it is important to confirm the association by family-based studies. We have genotyped the C1858T single nucleotide polymorphism in 410 affected sibpair and simplex families and found that the 1858T alleles were transmitted to 57.2% (152 of 209) of diabetic children. This significant distortion of transmission ($P < 0.003$) provides further support for the observed association in case/control studies. Association between *PTPN22* and type 1 diabetes has now been observed in two large North-American populations and one Italian population. The association is also confirmed in a large collection of diabetic families using the transmission disequilibrium test. After the manuscript was accepted, it has come to our attention that association between type 1 diabetes and *PTPN22* has also been demonstrated in a large U.K. cohort (18). Taken together, these results provided compelling evidence for a contribution of the PTPN22 gene to type 1 diabetes. Accumulating evidence also suggests that the *PTPN22* is a common susceptibility gene for autoimmune diseases. Significant association has now been reported for type 1 diabetes, rheumatoid arthritis, and systemic lupus erythematosus, and it will be important to analyze other autoimmune diseases.

The C1858T substitution changes the amino acid from arginine (620R) to tryptophan (620W) at codon 620. This residue resides in the P1 proline-rich motif that is involved in binding to the SH3 domain of Csk (9). In vitro experiments have showed that the mutant 620W LYP protein (1858T) does not bind Csk (14). Coimmunoprecipitation of Csk in 293 T-cells expressing tagged 620R and 620W isoforms also indicated that the 620R protein has approximately threefold-higher affinity than the 620W isoform (12). These results suggest that the mutant LYP 620W protein would result in higher T-cell activation. Direct assay on T-cell function would shed further light on how this mutation influences T-cell activation. Since LYP is also expressed in other immune cells, including NK cells, neutrophils, monocytes, and dentridic cells (12), it would be important to investigate whether LYP can influence autoimmunity through regulation of activation of these cells.

ACKNOWLEDGMENTS

This study was supported in part by a grant from the National Institute of Child Health and Development (2RO1HD37800) to J.-X.S.

The authors thank Dr. Robert Podolsky for helpful discussion.

REFERENCES

1. She JX, Marron MP: Genetic susceptibility factors in type 1 diabetes: linkage, disequilibrium and functional analyses. *Curr Opin Immunol* 10:682–689, 1998
2. Cox NJ, Wapelhorst B, Morrison VA, Johnson L, Pinchuk L, Spielman RS, Todd JA, Concannon P: Seven regions of the genome show evidence of linkage to type 1 diabetes in a consensus analysis of 767 multiplex families. *Am J Hum Genet* 69:820–830, 2001
3. Pugliese A: Genetics of type 1 diabetes. *Endocrinol Metab Clin North Am* 33:1–16, 2004
4. Risch N, Merikangas K: The future of genetic studies of complex human diseases. *Science* 273:1516–1517, 1996
5. Guo D, Li M, Zhang Y, Yang P, Eckenrode S, Hopkins D, Zheng W, Purohit S, Podolsky RH, Muir A, Wang J, Dong Z, Brusko T, Atkinson M, Pozzilli P, Zeidler A, Raffel LJ, Jacob CO, Park Y, Serrano-Rios M, Larrad MT, Zhang Z, Garchon HJ, Bach JF, Rotter JI, She JX, Wang CY: A functional variant of SUMO4, a new I kappa B alpha modifier, is associated with type 1 diabetes. *Nat Genet* 36:837–841, 2004
6. Barratt BJ, Payne F, Lowe CE, Hermann R, Healy BC, Harold D, Concannon P, Gharani N, McCarthy MI, Olavesen MG, McCormack R, Guja C, Ionescu-Tirgoviste C, Undlien DE, Ronningen KS, Gillespie KM, Tuomilehto-Wolf E, Tuomilehto J, Bennett ST, Clayton DG, Cordell HJ, Todd JA: Remapping the insulin gene/*IDDM2* locus in type 1 diabetes. *Diabetes* 53:1884–1889, 2004

7. Nistico L, Buzzetti R, Pritchard LE, Van der Auwera B, Giovannini C, Bosi E, Larrad MT, Rios MS, Chow CC, Cockram CS, Jacobs K, Mijovic C, Bain SC, Barnett AH, Vandewalle CL, Schuit F, Gorus FK, Tosi R, Pozzilli P, Todd JA: The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes: Belgian Diabetes Registry. *Hum Mol Genet* 5:1075–1080, 1996
8. Marron MP, Raffel LJ, Garchon HJ, Jacob CO, Serrano-Rios M, Martinez Larrad MT, Teng WP, Park Y, Zhang ZX, Goldstein DR, Tao YW, Beaurain G, Bach JF, Huang HS, Luo DF, Zeidler A, Rotter JI, Yang MC, Modilevsky T, Maclaren NK, She JX: Insulin-dependent diabetes mellitus (IDDM) is associated with CTLA4 polymorphisms in multiple ethnic groups. *Hum Mol Genet* 6:1275–1282, 1997
9. Cohen S, Dadi H, Shaoul E, Sharfe N, Roifman CM: Cloning and characterization of a lymphoid-specific, inducible human protein tyrosine phosphatase, Lyp. *Blood* 93:2013–2024, 1999
10. Hill RJ, Zozulya S, Lu YL, Ward K, Gishizky M, Jallal B: The lymphoid protein tyrosine phosphatase Lyp interacts with the adaptor molecule Grb2 and functions as a negative regulator of T-cell activation. *Exp Hematol* 30:237–244, 2002
11. Cloutier JF, Veillette A: Cooperative inhibition of T-cell antigen receptor signaling by a complex between a kinase and a phosphatase. *J Exp Med* 189:111–121, 1999
12. Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, Ardlie KG, Huang Q, Smith AM, Spoerke JM, Conn MT, Chang M, Chang SY, Saiki RK, Catanese JJ, Leong DU, Garcia VE, McAllister LB, Jeffery DA, Lee AT, Batliwalla F, Remmers E, Criswell LA, Seldin MF, Kastner DL, Amos CI, Sninsky JJ, Gregersen PK: A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am J Hum Genet* 75:330–337, 2004
13. Gjorloff-Wingren A, Saxena M, Williams S, Hammi D, Mustelin T: Characterization of TCR-induced receptor-proximal signaling events negatively regulated by the protein tyrosine phosphatase PEP. *Eur J Immunol* 29: 3845–3854, 1999
14. Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, MacMurray J, Meloni GF, Lucarelli P, Pellecchia M, Eisenbarth GS, Comings D, Mustelin T: A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat Genet* 36:337–338, 2004
15. Kyogoku C, Langefeld CD, Ortmann WA, Lee A, Selby S, Carlton VE, Chang M, Ramos P, Baechler EC, Batliwalla FM, Novitzke J, Williams AH, Gillett C, Rodine P, Graham RR, Ardlie KG, Gaffney PM, Moser KL, Petri M, Begovich AB, Gregersen PK, Behrens TW: Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. *Am J Hum Genet* 75:504–507, 2004
16. Luo DF, Buzzetti R, Rotter JI, Maclaren NK, Raffel LJ, Nistico L, Giovannini C, Pozzilli P, Thomson G, She JX: Confirmation of three susceptibility genes to insulin-dependent diabetes mellitus: IDDM4, IDDM5 and IDDM8. *Hum Mol Genet* 5:693–698, 1996
17. Luo DF, Bui MM, Muir A, Maclaren NK, Thomson G, She JX: Affected-sib-pair mapping of a novel susceptibility gene to insulin-dependent diabetes mellitus (IDDM8) on chromosome 6q25–q27. *Am J Hum Genet* 57:911–919, 1995
18. Smyth D, Cooper JD, Collins JE, Heward JM, Franklyn JA, Howson JM, Vella A, Nutland S, Rance HE, Maier L, Barratt BJ, Guja C, Ionescu-Tirgoviste C, Savage DA, Dunger DB, Widmer B, Strachan DP, Ring SM, Walker N, Clayton DG, Twells RC, Gough SC, Todd JA: Replication of an association between the lymphoid tyrosine phosphotase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. *Diabetes* 53:3020–3023, 2004