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Contribution of the R620W polymorphism of protein tyrosine phosphatase non-receptor 22 to systemic lupus erythematosus in Poland

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ABSTRACT

The protein tyrosine phosphatase non-receptor 22 (PTPN22) 1858 C>T polymorphic variant gene (rs2476601) displays an association with systemic lupus erythematosus (SLE) and other autoimmune diseases. However, its contribution to SLE has been found to be disputable. We therefore examined the association of PTPN22 1858 C>T polymorphism with susceptibility to SLE in the Polish population, among patients with SLE (n=150) and controls (n=300). We found a contribution of the PTPN22 1858 C>T polymorphism to the incidence of SLE. Women with the PTPN22 TT and PTPN22 CT genotypes displayed a 2.016-fold increased risk of SLE (95% CI=1.324 – 3.070, P=0.0014). However, we did not observe an increased risk for the homozygous PTPN22 TT genotype OR= 2.552 (95% CI=0.6748-9.64, p=0.1675). Our results confirm an association of the 1858 C>T polymorphism of the PTPN22 gene with SLE, which was previously observed in other populations.

Introduction

Systemic lupus erythematosus (SLE) is a complex disease characterized by loss of self-tolerance causing immune-mediated tissue destruction (1, 2). Defective function of $CD4^+$ T cells and abnormal activation of B cells result in autoantibody production and immune complex formation (3-5). SLE is a multifactorial disease caused by interactions of environmental and genetic factors (6-9). Involvement of prone genes in SLE etiology has been examined, and series of genes that encode proteins relevant for the function of immune cells have been considered as candidate susceptibility genes for SLE morbidity (7-9).

The protein tyrosine phosphatase non-receptor 22 (PTPN22) 1858 C>T polymorphic variant gene (rs2476601) displays an association with SLE and other autoimmune diseases (10, 11). The PTPN22 gene is located in chromosome 1p13.3-p13.1 and encodes lymphoid-specific phosphatase (Lyp) (12-15). Lyp is an intracellular protein tyrosine phosphatase, which uses a proline-rich motif to interact with the SH3 domain of the Csk kinase and inhibits kinases contributing to T-cell activation (12). The PTPN22 1858 C>T transition alters the amino acid at position 620 from highly conserved arginine (R) to tryptophan (W). This amino acid substitution is located in the proximal proline-rich SH3-binding domain (10, 12). The change from arginine to tryptophan disrupts the interaction of Lyp with Csk, thereby disturbing the regulation of the T cell receptor-signaling kinases, Lck, Fyn, and ZAP-70 (10-14).

It has been reported that the PTPN22 1858T allele contributes to numerous autoimmune diseases, including rheumatoid arthritis (14, 16-24), type 1 diabetes mellitus (25-27), and Grave's disease (27-29), as well as SLE (30-33). Despite the contribution of PTPN22 1858C>T to various autoimmune diseases, there are some autoimmune disorders in which PTPN22 1858 C>T does not seem to play a role in susceptibility (34-38). Moreover, contribution of the PTPN22 1858 C>T polymorphism in SLE development has been found to be controversial (39). Therefore, we investigated the prevalence of PTPN22 1858 C>T polymorphic variants in Polish patients with SLE.

Patients and methods

Patients and controls

One hundred and fifty patients (only women) diagnosed with SLE according to the American College of Rheumatology Classification (ACRC) criteria for systemic lupus erythematosus were chosen in a consecutive manner for studies at the Institute of Rheumatology in Warsaw, Poland (40, 41). The control group consisted of 300 healthy women of Polish Caucasian origin, recruited from individuals who had a comprehensive examination at the Institute of Mother and Child in Warsaw.

The protocol of the study was approved by the Local Ethical Committee of Poznań University of Medical Sciences. Written informed consent was obtained from all participating subjects.

Clinical manifestations were determined at the time of SLE diagnosis. Clinical manifestations of SLE in the patient group included central nervous system (seizure, psychosis, organic brain syndrome, visual disturbances,

Table I. Association of the PTPN22 polymorphisms in SLE patients and controls.

		n.	Genotype distribution absolute number (frequency %)			Allele absolute number (frequency %)			Odds ratio (95% CI)	P[c]
			C/C	T/C	T/T	C	T	P[c]		
PTPN22 1858T>C	Controls Total	300	227 (76)	69 (23)	4 (1)	523 (87)	77 (13)	0.0013	2.552 (0.6748-9.649)[a]	0.1675
	SLE Total	150	91 (61)	54 (36)	5 (3)	236 (79)	64 (21)		2.016 (1.324-3.070)[b]	0.0014

The odds ratio was calculated for [a]homozygous patients carrying risk allele *vs*. homozygous or heterozygous, [b]homozygous or heterozygous carrying risk allele *vs*. homozygous. [c]Fisher's exact test.

cranial nerve disorder, lupus headache) and vascular manifestations (vasculitis, Raynaud's phenomenon, thromboembolism). The renal and musculoskeletal manifestations encompassed urinary casts, hematuria, proteinuria, and pyuria, as well as myositis and arthritis, respectively. Pleurisy and pericarditis characterized serosal manifestations, and a new rash, alopecia, and mucosal ulcers were used to assess dermal clinical findings. Immunologic manifestations involved patients with a decrease of CH50, C3, or C4 below the lower limit, and with most of these patients possessing anti-double stranded DNA antibody (anti-dsDNA Ab). Constitutional and clinical findings included fever >38^{0}C, while hematologic findings encompassed thrombocytopenia and leucopenia.

The mean age of SLE patients at diagnosis was 35±14 years and for controls 36±11 years. The mean disease duration at the time of the study was 6±3 years. We also established that in our investigated group there was a positive SLE diagnosis in first or second degree relatives in approximately 2% of the SLE patients.

Clinical manifestations of SLE in the patient group include central nervous system (16%), vascular (16%), renal (57%), musculoskeletal (55%), serosal (19%), dermal (56%), immunologic (45%), fever (8%), and hematologic (38%) components.

Genotyping

DNA was isolated from peripheral blood lymphocytes by salt extraction. Polymorphic variants of PTPN22 1858C>T were identified using PCR with primer pair 5' TTTATTTTGCTTTTTCCTTGAATG 3' and 5' TCCTGGAAGAAAGTGAATATAG 3'; enzyme digestion followed the identification process. The PCR-amplified fragments of the PTPN22 that were 726bp in length were subjected to digestion with *RsaI* (GT/AC). The -1858T allele was cleaved into 466bp and 260bp fragments, whereas the 1858C allele was cleaved into 466bp, 216bp, and 44bp. DNA fragments were separated by electrophoresis on 3% agarose gel and visualized by ethidium bromide staining. Confirmation of polymorphism was performed by sequencing analysis.

Statistical analysis

The distribution of genotypes in all groups was evaluated for deviation from Hardy-Weinberg equilibrium. The Fisher's exact test was applied to examine differences in the genotypic and allelic distribution between patients and controls. The Odds Ratio (OR) and 95% Confidence Intervals were also calculated. A p-value <0.05 was considered statistically significant. Power analysis was performed using Fisher's exact test, which is available at an on-line internet service, http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize.

Results

Distribution of PTPN22 1858 C>T gene polymorphism in patients with SLE

Genotype analysis of the 1858 C>T polymorphism revealed no significant deviation form Hardy-Weinberg equilibrium in patient and control groups.

The prevalence of the T allele was 1.6-fold times higher in patients than in controls, and reached 21% and 13% in these groups, respectively (Table I). The frequencies of homozygous PTPN22 TT genotype in patients and controls were 3% and 1%, respectively (Table I). Moreover, the frequency of heterozygous PTPN22 CT in patients was also higher compared to controls, and amounted to 36% and 23% in these groups, respectively (Table I).

We found that women with the PTPN22 TT and PTPN22 CT genotypes displayed a 2.016-fold increased risk of SLE (95% CI=1.324 – 3.070, p=0.0014). However, we did not observe an increased risk for the homozygous PTPN22 TT genotype OR=2.552 (95% CI=0.6748-9.64, p=0.1675). The OR for allele frequency showed a 1.842-fold increased risk of SLE (95% CI=1.278 – 2.655, p =0.0013). The statistical power of this study amounted to 88% for the PTPN22 TT and PTPN22 CT genotypes and 21% for the PTPN22 TT genotype**.**

We did not observe a significant association between clinical manifestations of SLE and disease activity and prevalence of PTPN22 1858 C>T variants.

Discussion

Tyrosine phosphatases detach phosphate from tyrosine residues and regulate T cell signal transduction, which plays a crucial role in various aspects of T cell function (42). Alteration of tyrosine phosphatase functionality has been demonstrated in the pathogenesis

of human diseases (43). It has been suggested that disturbance in tyrosine phosphorylation in SLE T cells is linked to T cell effector dysfunction, which is observed in the immunopathogenesis of SLE (4, 44, 45).

Lyp is a tyrosine phosphatase, which has had its specific role in T-cell activation confirmed by observation of the threshold reduction for T-cell-receptor signaling in knock-out PTPN22 gene mice (15). Moreover, the functional significance of Lyp in negative regulation of T cells and its location in the chromosome region associated with SLE and rheumatoid arthritis (RA) susceptibility (46, 47) make it a good genetic marker for SLE and other autoimmune diseases. Experiments *in vitro* have revealed that the Lyp tryptophan variant binds less efficiently to Csk than the Lyp arginine variant. This suggests that T-cells expressing the T-allele may be hyperresponsive, and consequently, individuals carrying this allele could be susceptible to developing autoimmune disorders (13, 14).

In the present study, we found an association between the functional 1858 C>T polymorphism of the *PTPN22* gene and susceptibility to SLE. Our observations confirm the findings of Orozco *et al*., Kyogoku *et al*., and Reddy *et al*., who demonstrated an association of 1858 C>T polymorphism to SLE (30, 31, 33). In contrast, Wu *et al*. did not observe a contribution of the PTPN22 1858 C>T gene polymorphism in Caucasian SLE patients from northern America, the UK, or Finland (39). They found that this polymorphism can be a risk factor for comorbid autoimmune thyroid disease in patients with SLE (39). These contradictory reports can be explained by the findings of Kaufman *et al*., who discovered an association between the PTPN22 1858 T allele and familial SLE but not with sporadic SLE in Europeans and Americans (48). The differences in influence of the PTPN22 1858 C>T gene polymorphism on SLE morbidity may also result from other diseases and genetic heterogeneity that usually confound the investigation of complex multigenic diseases. Different environmental factors together with genetic heterogeneity may also affect the impact that the PTPN22 1858 C>T polymorphism has on SLE incidence (6). It is possible that other polymorphisms in linkage disequilibrium with PTPN22 1858 C>T may be responsible for the contribution to the development of SLE.

However**,** to more precisely determine the contribution of the PTPN22 tryptophane variant to SLE morbidity, further investigation of the prevalence of these variants in other populations is needed.

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References

1. SEKIGAWA I, NAITO T, HIRA K *et al*.: Possible mechanisms of gender bias in SLE: a new hypothesis involving a comparison of SLE with atopy. *Lupus* 2004; 13: 217-22.
2. BOMBARDIER C, GLADMAN DD, UROWITZ M.B, CARON D, CHANG CH: Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992; 35: 630-40.
3. STOHL W, METYAS S, TAN SM *et al*.: B lymphocyte stimulator overexpression in patients with systemic lupus erythematosus: longitudinal observations. *Arthritis Rheum* 2003; 48: 3475-86.
4. TSOKOS GC, LIOSSIS SN: Immune cell signaling defects in lupus: activation, anergy and death. *Immunol Today* 1999; 20: 119-124.
5. JANUCHOWSKI R, WUDARSKI M, CHWALIŃSKA-SADOWSKA H, JAGODZINSKI PP: Prevalence of ZAP-70, LAT, SLP-76, and DNA methyltransferase 1 expression in CD4(+) T cells of patients with systemic lupus erythematosus. *Clin Rheumatol* 2007; 27: 21-7.
6. LOVE LA: New environmental agents associated with lupus-like disorders. *Lupus* 1994; 3: 467-71.
7. WONG M, TSAO BP: Current topics in human SLE genetics. *Springer Semin Immunopathol* 2006; 28: 97-107.
8. AL-AWADHI AM, HAIDER MZ, SHARMA PN *et al*.: Angiotensin-converting enzyme gene polymorphism in Kuwaiti patients with systemic lupus erythematosus. *Clin Exp Rheumatol* 2007; 25: 437-42.
9. BURZYNSKI M, DURIAGIN S, MOSTOWSKA M, WUDARSKI M, CHWALINSKA-SADOWSKA H, JAGODZINSKI PP: MTR 2756 A>G polymorphism is associated with the risk of systemic lupus erythematosus in the Polish population. *Lupus* 2007; 16: 450-4.
10. SIMINOVITCH KA: PTPN22 and autoimmune disease. *Nat Genet* 2004; 36: 1248-9.
11. CHUNG SA, CRISWELL LA: PTPN22: its role in SLE and autoimmunity. *Autoimmunity* 2007; 40: 582-90.
12. COHEN S, DADI H, SHAOUL E, SHARFE N, ROIFMAN CM: Cloning and characterization of a lymphoid-specific, inducible human protein tyrosine phosphatase. *Lyp Blood* 1999; 93: 2013-24.
13. 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: 337–38.
14. 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: 330-37.
15. HASEGAWA K, MARTIN F, HUANG G, TUMAS D, DIEHL L, CHAN AC: PEST domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory T cells. *Science* 2004; 303: 685-9.
16. 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: 1694-9.
17. 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; 52:1993-8.
18. CARLTON VE, HU X, CHOKKALINGAM AP *et al*.: PTPN22 Genetic variation: evidence for multiple variants associated with rheumatoid arthritis. *Am J Hum Genet* 2005; 77: 567-81.
19. SELDIN MF, SHIGETA R, LAIHO K *et al*.: Finnish case-control and family studies support PTPN22 R620W polymorphism as a risk factor in rheumatoid arthritis, but suggest only minimal or no effect in juvenile idiopathic arthritis. *Genes Immun* 2005; 6: 720-2.
20. SIMKINS HM, MERRIMAN ME, HIGHTON J *et al*.: Association of the PTPN22 locus with rheumatoid arthritis in a New Zealand Caucasian cohort. *Arthritis Rheum* 2005; 52: 2222–5.
21. GOMEZ LM, ANAYA JM, GONZALEZ CI *et al*.: PTPN22 C1858T polymorphism in Colombian patients with autoimmune diseases. *Genes Immun* 2005; 6: 628-31.
22. WESOLY J, VAN DER HELM-VAN MIL AH, TOES RE *et al*.: Association of the PTPN22 C1858T single-nucleotide polymorphism with rheumatoid arthritis phenotypes in an inception cohort. *Arthritis Rheum* 2005; 52: 2948-50.
23. STEER S, LAD B, GRUMLEY JA, KINGSLEY GH, FISHER SA: Association of R602W in a protein tyrosine phosphatase gene with a high risk of rheumatoid arthritis in a British population: evidence for an early onset/disease severity effect. *Arthritis Rheum* 2005; 52: 358-60.
24. ZHERNAKOVA A, EERLIGH P, WIJMENGA C, BARRERA P, ROEP BO, KOELEMAN BP: Differential association of the PTPN22 coding variant with autoimmune diseases in a Dutch population. *Genes Immun* 2005; 6: 459-61.
25. 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: 337-8.
26. ZHENG W, SHE JX: Genetic association between a lymphoid tyrosine phosphatase (PTPN22) and type 1 diabetes. *Diabetes* 2005; 54: 906-8.
27. 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: 3020-3.
28. VELAGA MR, WILSON V, JENNINGS CE *et al*.: The codon 620 tryptophan allele of the lymphoid tyrosine phosphatase (LYP) gene is a major determinant of Graves' disease. *J Clin Endocrinol Metab* 2004; 89: 5862-5.
29. SKORKA A, BEDNARCZUK T, BAR-ANDZIAK E, NAUMAN J, PLOSKI R: Lymphoid tyrosine phosphatase (PTPN22/LYP) variant and Graves' disease in a Polish population: association and gene dose-dependent correlation with age of onset. *Clin Endocrinol* 2005; 62: 679-82.
30. OROZCO G, SÁNCHEZ E, GONZÁLEZ-GAY MA *et al*.: Association of a functional single-nucleotide polymorphism of PTPN22, encoding lymphoid protein phosphatase, with rheumatoid arthritis and systemic lupus erythematosus. *Arthritis Rheum* 2005; 52: 219-24.
31. 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: 504-7.
32. VIKEN MK, AMUNDSEN SS, KVIEN TK *et al*.: Association analysis of the 1858C > T polymorphism in the PTPN22 gene in juvenile idiopathic arthritis and other autoimmune diseases. *Genes Immun* 2005; 6: 271-3.
33. REDDY MV, JOHANSSON M, STURFELT G *et al*.: The R620W C/T polymorphism of the gene PTPN22 is associated with SLE independently of the association of PDCD1. *Genes Immun* 2005; 6: 658-62.
34. MARTÍN MC, OLIVER J, URCELAY E *et al*.: The functional genetic variation in the PTPN22 gene has a negligible effect on the susceptibility to develop inflammatory bowel disease. *Tissue Antigens* 2005; 66: 314-7.
35. WAGENLEITER SE, KLEIN W, GRIGA T, SCHMIEGEL W, EPPLEN JT, JAGIELLO P: A case-control study of tyrosine phosphatase (PTPN22) confirms the lack of association with Crohn's disease. *Int J Immunogenet* 2005; 32: 323-4.
36. MATESANZ F, RUEDA B, OROZCO G *et al*.: Protein tyrosine phosphatase gene (PTPN22) polymorphism in multiple sclerosis. *J Neurol* 2005; 252: 994-5.
37. ITTAH M, GOTTENBERG JE, PROUST A *et al*.: No evidence for association between 1858 C/T single-nucleotide polymorphism of PTPN22 gene and primary Sjogren's syndrome. *Genes Immun* 2005; 6: 457-8.
38. GONZALEZ-GAY MA, OLIVER J, OROZCO G, GARCIA-PORRUA C, LOPEZ-NEVOT MA, MARTIN J: Lack of association of a functional single nucleotide polymorphism of PTPN22, encoding lymphoid protein phosphatase, with susceptibility to biopsy-proven giant cell arteritis. *J Rheumatol* 2005; 32: 1510-2.
39. WU H, CANTOR RM, GRAHAM DS *et al*.: Association analysis of the R620W polymorphism of protein tyrosine phosphatase PTPN22 in systemic lupus erythematosus families: increased T allele frequency in systemic lupus erythematosus patients with autoimmune thyroid disease. *Arthritis Rheum* 2005; 5: 2396-402.
40. TAN EM, COHEN AS, FRIES JF *et al*.: The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; 25: 1271-7.
41. HOCHBERG MC: Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
42. MUSTELIN T, ALONSO A, BOTTINI N *et al*.: Protein tyrosine phosphatases in T cell physiology. *Mol Immunol* 2004; 41: 687-700.
43. MUSTELIN T, VANG T, BOTTINI N: Protein tyrosine phosphatases and the immune response. *Nat Rev Immunol* 2005; 5: 43-57.
44. KAMMER GM, PERL A, RICHARDSON BC, TSOKOS GC: Abnormal T cell signal transduction in systemic lupus erythematosus. *Arthritis Rheum* 2002; 46: 1139-54.
45. TSOKOS GC, NAMBIAR MP, TENBROCK K, JUANG YT: Rewiring the T-cell: signalling defects and novel prospects for the treatment of SLE. *Trends* Immunol 2003; 259-63.
46. JAWAHEER D, SELDIN MF, AMOS CI *et al*., NORTH AMERICAN RHEUMATOID ARTHRITIS CONSORTIUM: Screening the genome for rheumatoid arthritis susceptibility genes: a replication study and combined analysis of 512 multicase families. *Arthritis Rheum* 2003; 48: 906-16.
47. GAFFNEY PM, KEARNS GM, SHARK KB *et al*.: A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pair families. *Proc Natl Acad Sci USA* 1998; 95: 14875-9.
48. KAUFMAN KM, KELLY JA, HERRING BJ *et al*.: Evaluation of the genetic association of the PTPN22 R620W polymorphism in familial and sporadic systemic lupus erythematosus. *Arthritis Rheum* 2006; 54: 2533-40.