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Single Nucleotide Polymorphism rs 2476601 of PTPN22 Gene and Susceptibility to Rheumatoid Arthritis in Iranian Population

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ABSTRACT

The rs2476601 (R620W, C1858T) polymorphism in PTPN22 gene has been repeatedly reported to be associated with rheumatoid arthritis (RA). The rs 2476601 is widely suggested for predictive testing and risk assessment for RA. The aim of this study was to test the possible association of this SNP with RA in Iranian population.

A total of 872 samples (405 confirmed RA patients and 467 healthy controls) were recruited in this study. Genomic DNA was extracted from whole blood and the genotyping was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). Genotyping for a set of samples were re-confirmed by two other rounds of genotyping, using another PCR-RFLP experiment with different enzyme and DNA sequencing.

All 872 samples were genotyped as homozygous CC in first round of genotyping. Genotyping was repeated for 30% of samples by another restriction enzyme and for 10% of samples by sequencing. Again all samples showed homozygous CC genotype.

This study suggests that the rs2476601 polymorphism of PTPN22 gene is mono-morphic in Iranian population, containing only C allele.

Considering that previous studies in other populations reported the T allele as the risk allele at this locus, the present study concluded that rs2476601 play no role in susceptibility to RA and other autoimmune diseases in Iranian population. This finding has significant future clinical implications in determining the strategy for risk assessment and predictive testing for such diseases in Iranian population.

Key words: Association Study; Genetic susceptibility; Protein Tyrosine Phosphatase; Non-Receptor Type 22; Rheumatoid Arthritis; Single Nucleotide Polymorphism

INTRODUCTION

Rheumatoid arthritis (RA) is known as one of the most common autoimmune diseases. It is an inflammatory, autoimmune systemic disease, and is characterized by the chronic inflammation and progressive destruction

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of the synovial joint, occasionally with systemic involvement that leads to disability and joint damage.[1] There is a remarkable consistency in the overall prevalence of RA in most adult population worldwide (~1%). The etiology of RA, like other autoimmune disorders is not fully understood; however, RA is complex disorder that interaction of genetic and environmental factor contribute to susceptibility. The genetic contribution to a susceptibility to RA is confirmed from studies of twins,[2] families[3] and in genome-wide linkage scans [4] which determine heritability estimate of 50-60% and suggest genetic component has a significant impact on disease susceptibility. The major genetic risk factors that have been reproducibly shown strong association with susceptibility are Human leukocyte antigen of DRB1 alleles Human leukocyte antigen of DRB1 alleles (HLA-DRB1), and Protein tyrosine phosphatases non-receptor 22 (PTPN22) genes.[5] Particular alleles of HLA-DRB1 gene account for 30-50% of overall genetic susceptibility to RA.[6]

After the first report of association between the PTPN22 Arg 620 Trp (R 620W) variant (rs2476601) and type 1 diabetes,[7] this variant emerged as the strongest common genetic risk factor for human autoimmunity outside the major histocompatability complex.[8]

The PTPN22 gene, maps to chromosome 1p13 and encodes a lymphoid-specific phosphatase known as LYP. LYP is an intracellular PTP which contains an N-terminal catalytic conserved domain and non catalytic C-terminus with 4 proline-rich domains and is involved in down regulation of T-cell activation through physical interaction of its proline-rich motif (referred to as P1) to the SH3 domain of C-terminal Src kinase (Csk). The rs2476601 functional polymorphism of PTPN22 (1858C>T) lies at the P1 motif, substitution of Trp for Arg at amino acid 620 (R620W) disrupts the interaction between LYP and Csk[8] and TCR signaling has been reduced in T cells containing mutated T allele. Surprisingly, the R620W substitution is a gain-of-function mutation and generates a more active phosphatase that acts as a more potent inhibitor in TCR signaling than the wild-type enzyme.[9] The effect of these structural changes seems to be an increase in the threshold level of stimulation required for TCR signaling. It was speculated that this activated mutation in LYP may cause a predisposition to autoimmune diseases either by failure to delete auto-reactive T cells or due to insufficient activity of regulatory T cells.[10]

Having the above findings, the aim of the current study was to determine the association between the rs2476601 of PTPN22 gene and RA susceptibility in Iranian population. This work is a part of a larger study evaluating the possibility of using different genetic variations as predictive markers in risk assessment for RA in Iranian population.

PATIENTS AND METHODS

The study was designed as a case-control population-based association study. In total 872 individuals (405 cases and 467 controls) participated in the study. All participants were of Iranian origin and gave informed written consent before entering to the study. All patients had been diagnosed with RA according to the 1987 American College of Rheumatology criteria for RA.[11] Besides that, the history of autoimmune diseases is one of exclusion criteria in selection of case and control groups. The control group was matched with the case group through their race and demographic features and the differences were not statistically significant. The demographic features of cases and controls are shown in the previous article.[12] The study was approved by the ethics committees at the Tehran University of Medical Sciences in terms of ethical and legal requirements. The experiments were performed in the genetic laboratories at the Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences.

Single Nucleotide Polymorphism (SNP) Genotyping

Whole blood was collected in ethylenediamine tetra acetic acid (EDTA) containing tubes. Genomic DNA was isolated, using standard phenol/chlorophorm extraction method.[13]

Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique and confirmed by direct DNA sequencing. The forward primer 5`-TCA CCA GCT TCC TCA ACC ACA-3` and the reverse primer 5`-GAT AAT GTT GCT TCA ACG GAA TTTA-3` were used as described by Bottini et al.[7] The polymerase chain reaction (PCR) was carried out in a total volume of 25µl containing 100-200 ng of genomic DNA, 0.5 mM of each primer, 2 mM MgCl2, 0.2 mM dNTP, and 2.5 units Taq DNA polymerase.

Amplification was performed with a denaturation step at 95°C for 2 min, followed by 35 cycles of

denaturation at 95°C for 1 min, annealing at 60°C for 30 s, extension at 72°C, and the final extension at 72°C for 5 min. 3-5 µl of PCR products were run on agarose gel to see the target band before setting the restriction digestion reaction. Digestion was performed in a total volume of 20 µl, containing 15 µl of PCR products, 5 units of RsaI (10 Units/µl, Fermentas Inc.) or Xcm I (5 Units/µl, New England Biolabs Inc.), 2 µl 10x enzyme buffer, and 2.5 or 2 µl of ddH_2o. Digestion reaction was incubated at 37°C overnight.

The amplified PCR fragment had a size of 215 bp (Figure 1). The RsaI restriction enzyme recognizes the -5'-…GTAC site…-3'. Therefore, only amplified fragments with C allele at rs2476601 locus were cut with enzyme resulting in two fragments of 42 and 173 bp (Figure.3). On the other hand, the Xcm I recognition site is 5'-…CCANNNNNNNNNTGG…-3'. Hence, only amplified fragments with T allele at rs2476601 locus were cut with enzyme producing two fragments of 46 and 169 bp.

Products of restriction digestion were run on 3% agarose gel next to an appropriate DNA size marker, stained by ethidium bromide, visualized under UV light and photographed.

Direct sequencing of PCR products was performed by 3730xl DNA Analyzer (Applied Biosystems) using big dye terminator.

RESULTS

In total, 872 samples (405 patients and 467 controls) were analyzed for the rs2476601 of PTPN22 gene. The first round of genotyping for all samples was performed by PCR-RFLP using RsaI enzyme. All samples were genotyped as homozygous CC, Producing two fragments of 173 and 42 bp after restriction digestion and there was neither CT nor TT genotypes (Figure 2, and Table 1).

To confirm this finding, around 30% of samples (250 samples) were re-genotyped by another PCR-RFLP using XcmI enzyme. All samples showed CC genotype, appeared by only one band of 215 bp in agarose gel after digestion and there was neither CT nor TT genotype (Figure 4).

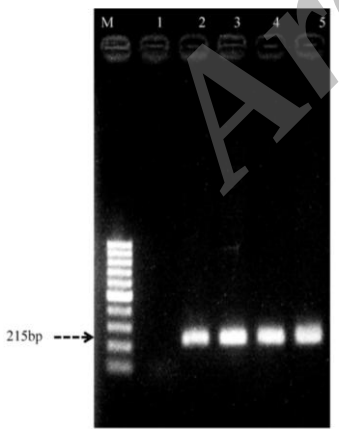

Figure 1. PCR products from various gDNA samples. M: 100-bp DNA ladder, lane 1: negative control, lane 2 to 5 PCR products

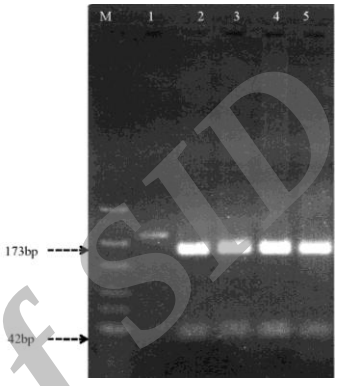

Figure 2. Digestion of PCR products with Rsa1 restriction Enzyme. Lane M contains ultra low range ladder from Fermentase. Lane1: negative control (without enzyme), lane 2-3 represents PCR products digested with Rsa1 in two cases and lane 4-5 show PCR products of controls digested with Rsa1. All samples have CC genotype (GG on the complementary strand).

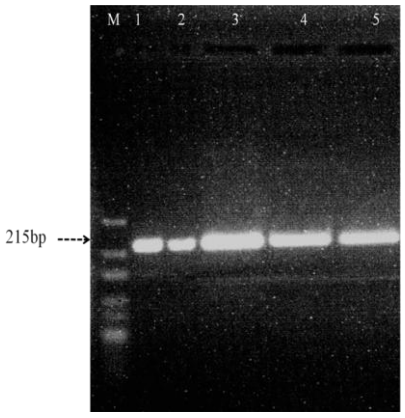

Figure 3. Digestion of PCR product with XcmI restriction Enzyme. Lane M contains ultra low range ladder from Fermentase. Lane1 contains PCR product only, lane 2 negative control (without enzyme), lane 3-5 represents case 1, case 2 and control 1, respectively digested with XcmI enzyme. All samples have CC genotype (GG on the complementary strand).

Table 1. PTPN22 rs2476601 genotype frequencies in RA patients and control subjects

Rs2476601 (C1858T)	CC	CT	TT	Total
Cases	405	0	0	405
Controls	467	0	0	467

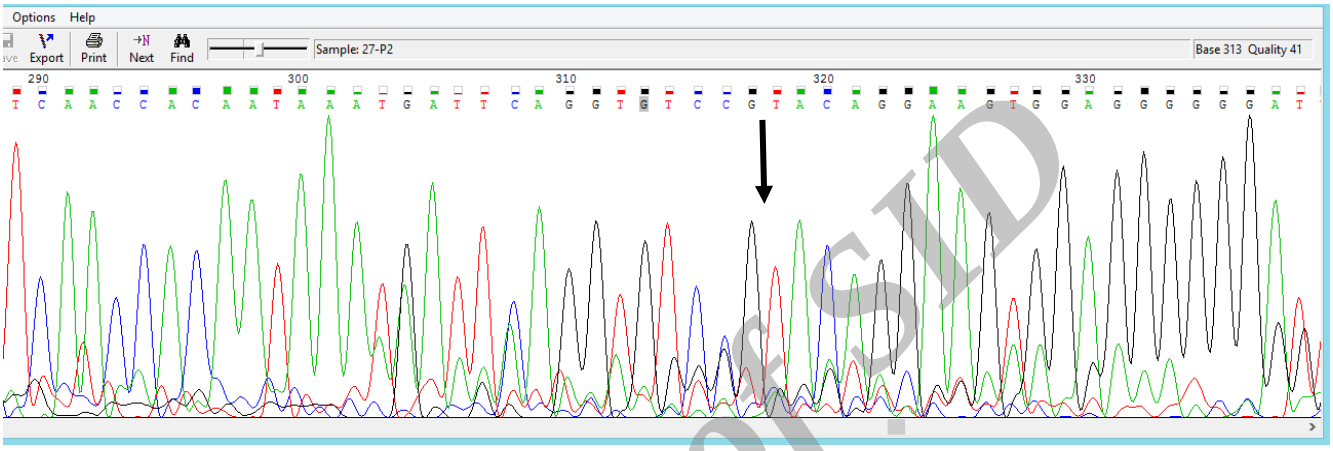

Figure 4. Sequencing result for rs2476601. Chromatogram shows homozygote GG (CC on complementary strand) genotype for rs2476601 of PTPN22 gene.

Nearly 10% of samples (85 samples) were re-genotyped by direct sequencing of PCR products. All samples showed only C allele. The T allele was not detected in our study samples, suggesting that the rs2476601 is a mono-morphic site with only C allele in Iranian population (Figure 4).

DISCUSSION

The rs2476601 is a functional polymorphism of the PTPN22. It has been identified as a major common risk factor for several autoimmune disorders besides RA and Type 1 Diabetes, including Systemic lupus erythmatosus,[14] Juveline idiopathic arthritis,[15] Graves disease [16] generalized vitiligo,[17] Hashimato thyroiditis [18], Mysthenia gravis [19], systemic sclerosis [20] and Addison`s disease.[21] In addition, recent meta-analysis of the RA Genome Wide association Studies (GWAS) dataset indicate that rs2476601 of PTPN22 associates with susceptibility to RA.[22]

Although the risk allele for this SNP (T allele) was the same in different association studies, the geographic differences in allele frequency were a major finding. The frequency of T allele was reported approximately 12% in North Europe, while it is estimated around 6% in Southern Europe.[23] Interestingly, this polymorphism is extremely rare in East Asian and African-American populations.[24-27]

The different allele frequency of T allele is very important in determining the population attributable risk of this allele for the autoimmune diseases in different populations. It also affects any suggested screening or predictive testing protocol for these diseases. For example, a population attributable risk of up to 8% was reported for rs2476601 in different populations of European decent for RA.[28] Genotyping of this SNP is also suggested as a part of predictive testing strategy for RA beside HLA-SE genotyping and Anti-cyclic Citrullinated Peptide Antibodies (anti-CCP) measurement in serum.[28] The combination of HLA-SE and the PTPN22 1858T allele (rs2476601 T allele) gave sensitivity of 24.1% and specificity of 92.9% in European population.[28] On the other hand, adding positive serum anti-CCP Antibodies to the combination

HLA-SE and PTPN22 1858T allele showed a sensitivity of 22.1%, specificity of 100%, and Odds Ratio (OR) of 132.03.[12]

The absence of risk allele T in the gene pool of East Asians undermines the importance of this locus as a susceptibility locus for the RA and other autoimmune diseases. Having this, there is no basis for having rs2476601 genotyping in any predictive or screening protocol for such diseases in these populations.

Admittedly, in three separate studies on leprosy, RA and tuberculosis, no homozygous TT genotype was seen for rs2476601 of PTPN22.[29-31] There are many reasons in this regard. First reason is incomplete digestion of one restriction enzyme which they used in their studies. Moreover if they have CT genotype in their gene pool, they should find at least one TT genotype. However, many of our samples were genotyped with two restriction enzymes and there was not any evidence of incomplete digestion of restriction enzyme, also 10% of the samples including both cases and controls were sequenced and interestingly showed that there was no T allele in the gene pool of Iranian population, similar to other Asians/East Asians populations. This finding suggests that rs2476601 does not play a role in individual variation in susceptibility to RA and other autoimmune diseases in Iranian population and therefore suggests no place in future risk assessment strategy for these diseases.

In addition to such clinical applications of this finding for Iranian population, it may gain socio-historical and evolutionary implications. Presence of T allele in Turkish population[32] and increasing frequency of this allele towards North European populations are very interesting, suggesting possible origin of T allele in North Europe.

In conclusion, the rs2476601 of PTPN22 gene is a mono-morph locus, having only C allele in Iranian population; also this SNP plays no role in causing susceptibility to RA and other autoimmune diseases in Iranian population.

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REFERENCES

1. Klareskog L, Catrina AI, Paget S. Rheumatoid arthritis. Lancet. 2009;373(19157532):659-72.
2. MacGregor AJ, Snieder H, Rigby AS, Koskenvuo M, Kaprio J, Aho K, et al. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheum. 2000;43(10643697):30-7.
3. Bali D, Gourley S, Kostyu DD, Goel N, Bruce I, Bell A, et al. Genetic analysis of multiplex rheumatoid arthritis families. Genes Immun. 1999;1(11197302):28-36.
4. Etzel CJ, Chen WV, Shepard N, Jawaheer D, Cornelis F, Seldin MF, et al. Genome-wide meta-analysis for rheumatoid arthritis. Hum Genet. 2006;119(16612613):634-41.
5. Begovich AB, Carlton VEH, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, 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(15208781):330-7.
6. Imboden JB. The immunopathogenesis of rheumatoid arthritis. Annu Rev Pathol. 2009;4(18954286):417-34.
7. 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 I diabetes. Nat Genet. 2004;36(15004560):337-8.
8. Gregersen PK. Gaining insight into PTPN22 and autoimmunity. Nat Genet. 2005;37(16314859):1300-2.
9. Fiorillo E, Orrú V, Stanford SM, Liu Y, Salek M, Rapini N, et al. Autoimmune-associated PTPN22 R620W variation reduces phosphorylation of lymphoid phosphatase on an inhibitory tyrosine residue. J Biol Chem. 2010 Aug/20;285(34):26506-18.
10. 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(16273109):1317-9.
11. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988;31(3358796):315-24.
12. Ahmadlou S, Akhiani M, Salimzadeh A, Keramatipour M. Lack of Association between Single Nucleotide Polymorphism rs10818488 in TRAF1/C5 Region and Rheumatoid Arthritis in

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Iranian Population. Iranian Journal of Allergy, Asthma and Immunology. 2013;13(1):19-25.
13. Dracopoli L. Nicolas C. Extraction and precipitation of DNA. Curr Protoc Hum Genet. 2001;Appendix 3(18428221).
14. Kyogoku C, Langefeld CD, Ortmann WA, Lee A, Selby S, Carlton VEH, et al. Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. Am J Hum Genet. 2004;75(15273934):504-7.
15. Viken MK, Amundsen SS, Kvien TK, Boberg KM, Gilboe IM, Lilleby V, 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(15759012):271-3.
16. Velaga MR, Wilson V, Jennings CE, Owen CJ, Herington S, Donaldson PT, 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(15531553):5862-5.
17. Canton I, Akhtar S, Gavalas NG, Gawkrodger DJ, Blomhoff A, Watson PF, et al. A single-nucleotide polymorphism in the gene encoding lymphoid protein tyrosine phosphatase (PTPN22) confers susceptibility to generalised vitiligo. Genes Immun. 2005;6(16015369):584-7.
18. Criswell LA, Pfeiffer KA, Lum RF, Gonzales B, Novitzke J, Kern M, 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(15719322):561-71.
19. Vandiedonck C, Capdevielle C, Giraud M, Krumeich S, Jais J-P, Eymard B, et al. Association of the PTPN22*R620W polymorphism with autoimmune myasthenia gravis. Ann Neurol. 2006;59(16437561):404-7.
20. Dieude P, Guedj M, Wipff J, Avouac J, Hachulla E, Diot E, et al. The PTPN22 620W allele confers susceptibility to systemic sclerosis: findings of a large case-control study of European Caucasians and a meta-analysis. Arthritis Rheum. 2008;58(18576360):2183-8.
21. Skinningsrud B, Husebye ES, Gervin K, Lovas K, Blomhoff A, Wolff AB, et al. Mutation screening of PTPN22: association of the 1858T-allele with Addison's disease. Eur J Hum Genet. 2008;16(18301444):977-82.
22. Song G, Bae S, Lee Y. Pathway analysis of genome-wide association studies on rheumatoid arthritis. Clinical and experimental rheumatology. 2012;31(4):566-74.
23. Nagy ZB, Csanad M, Toth K, Borzsonyi B, Demendi C, Rigo J, et al. Current concepts in the genetic diagnostics of rheumatoid arthritis. Expert Rev Mol Diagn. 2010;10(20629510):603-18.
24. Ikari K, Momohara S, Inoue E, Tomatsu T, Hara M, Yamanaka H, et al. Haplotype analysis revealed no association between the PTPN22 gene and RA in a Japanese population. Rheumatology (Oxford). 2006;45(16690758):1345-8.
25. Kochi Y, Suzuki A, Yamada R, Yamamoto K. Genetics of rheumatoid arthritis: underlying evidence of ethnic differences. J Autoimmun. 2009;32(19324521):158-62.
26. Mori M, Yamada R, Kobayashi K, Kawaida R, Yamamoto K. Ethnic differences in allele frequency of autoimmune-disease-associated SNPs. J Hum Genet. 2005;50(15883854):264-6.
27. Zhang ZH, Chen F, Zhang XL, Jin Y, Bai J, Fu SB. PTPN22 allele polymorphisms in 15 Chinese populations. Int J Immunogenet. 2008;35(19046301):433-7.
28. Rodriguez-Rodriguez L, Lamas JR, Varade J, Tornero-Esteban P, Abasolo L, de la Concha EG, et al. Combined influence of genetic and environmental factors in age of rheumatoid arthritis onset. Rheumatol Int. 2011 (21922340).
29. Hashemi M, Atabaki M, Daneshvar H, Zakeri Z, Eskandari-Nasab E. Association of PTPN22 rs2476601 and EGFR rs17337023 Gene polymorphisms and rheumatoid arthritis in Zahedan, Southeast Iran. International journal of immunogenetics. 2013;40(4):299-305.
30. Aliparasti MR, Almasi S, Majidi J, Zamani F, Khoramifar AR, Azari ARF. Protein tyrosine phosphatase non-receptor type 22 gene polymorphism C1858T is not associated with leprosy in Azerbaijan, Northwest Iran. Indian journal of human genetics. 2013;19(4):403.
31. Kouhpayeh H, Hashemi M, Hashemi S, Moazeni-Roodi A, Naderi M, Sharifi-Mood B, et al. R620W functional polymorphism of protein tyrosine phosphatase non-receptor type 22 is not associated with pulmonary tuberculosis in Zahedan, southeast Iran. Genetics and Molecular Research. 2012;11(2):1075-81.
32. Ates A, Karaaslan Y, Karatayli E, Ertugrul E, Aksaray S, Turkyilmaz A, et al. Association of the PTPN22 gene polymorphism with autoantibody positivity in Turkish rheumatoid arthritis patients. Tissue Antigens. 2011;78(21506938):56-9.