Original Article

Assessment of protein tyrosine phosphatases number 22 polymorphism prevalence among rheumatoid arthritis patients: A study on Iranian patients

Mansour Salesi, Golshan Taghipour Boroujeni[1], Mansoor Salehi[2], Hadi Karimzadeh

Division of Rheumatology, [1]Division of Internal Medicine, [2]Division of Genetics, Medical School, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Background: It has been proposed that Trp (620) allotype of protein tyrosine phosphatases number 22 (PTPN22) gene can intensify the susceptibility to rheumatoid arthritis (RA) and other autoimmune diseases. Thus, in this study, the prevalence of this polymorphism has been surveyed among RA patients compared with healthy persons. The samples were selected from Isfahan province (one of the most populated area of Iran).

Materials and Methods: In this study, 100 patients (case group) and 100 healthy persons (control group) were participated voluntarily. The case group was selected from people who had referred to the rheumatology clinic of AlZahra University Hospital to follow-up their treatment and change their drugs dosage. The control group members, who were living in Isfahan province, mutually had similar age with patients. On a total, 22% of the case group was male and 75% of the control group was female. DNA was extracted from the blood sample of all cases and controls and the PTPN22 single nucleotide polymorphism (SNP) C1858> T gene polymorphism were studied using the polymerase chain reaction-restriction fragment length polymorphism method.

Results: PTPN22 SNP C1858> T gene polymorphism was observed in 11 persons (11%) of the case group and 8 persons (8%) of the control group.

Conclusion: The results show that the difference was not statistically significant in Isfahan RA population ($P = 0.47$; OR = 1.42; 95% CI 0.55-3.69). Although, another study on Iranian population had shown that this polymorphism confers susceptibility to RA.

Key Words: Isfahan, polymorphism, protein tyrosine phosphates number 22, rheumatoid arthritis

Address for correspondence:
Dr. Golshan Tahgipour Boroujeni, Division of Internal Medicine, Medical School, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: golshan.taghipour@gmail.com
Received: 23.03.2013, **Accepted:** 07.05.2013

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.143294

INTRODUCTION

Rheumatoid arthritis (RA) is a multi-system disease that the cause of disease has not been clarified yet. This disease is the most common form of chronic inflammatory arthritis, which often causes injury to joint and other body organs. RA disease entangles nearly 0.5-1% of adult all over the world and its

How to cite this article: Salesi M, Boroujeni GT, Salehi M, Karimzadeh H. Assessment of protein tyrosine phosphatases number 22 polymorphism prevalence among rheumatoid arthritis patients: A study on Iranian patients. Adv Biomed Res 2014;3:218.

prevalence differ from one place to other. Assessments show that genetic factors play a significant role in the onset and severity of the disease. Although their importance still is not clear still, there is an uncertainty about the amount of importance of their effect. Kurkó, *et al*. had a comprehensive review on genetic role in heritability of RA.[1] Malaise, *et al*. believed that RA is a syndrome, rather than a disease, which genetic is one of the most influences.[2] The alleles, which are related to the maximum rate of disease onset are at main histocompatibility complex (MHC) and it is shown that one third of genetic risk of disease onset places at this location. The genome wide association study (GWAS) has discovered that several genes at other place than MHC, which are related to RA sensitivity.

GWAS studies are on the basis of single nucleotide polymorphism that permits to analyze genetic structure of the complicated disease like RA. One of the best examples of non-MHC genes related to RA onset is non-receptor protein tyrosine phosphates number 22 (PTPN22). This gene encodes lymphoid tyrosine phosphatase that regulates B and T-cell function. Inheritance of PTPN22 gene can overact the proteins, which supposed to have influence on pathologic selection of B and T-cell. RA patients who have PTPN22 polymorphism solely suffer from positive anti-cyclic citrullinated peptide antibody (anti-CCP). Song, *et al*. showed that PTPN22 polymorphism confers susceptibility to RA in populations with different ethnicities, especially in European.[3]

The prevalence of this polymorphism is variable among European population from 3% to 10%, whereas it has not been recorded from Asian population.[4] Totaro, *et al*. evaluated the association between the PTPN22 rs2476601 polymorphism and RA in Italian subjects (396 RA cases and 477 controls). The PTPN22 T1858 allele was significantly more frequent in RA patients compared to controls. Their result showed that frequency in Italian RA patients was lower than the one described in northern European populations.[5] In a survey, which has carried out by Majorczyk, *et al*. on Polish population, on 173 RA patients and 180 normal controls, significance difference was observed in PTPN22 polymorphism between two groups.[6] Furthermore, in a study by Sahin, *et al*. it was observed that there is no relationship between PTPN22 gene polymorphism and RA disease. In this research, 167 Turkish person with RA disease and 177 normal controls were screened by polymerase chain reaction (PCR).[7] Another survey by Hinks, *et al*. showed some similarities between juvenile RA disease and RA disease in relation of Human Leukocyte Antigen DR subregion 4 (HLA-DR4) and PTPN22 polymorphisms.[8]

An investigation by Balsa, *et al*. showed that the homozygote and heterozygote carriers of PTPN22 1858T have less chance for disease remission.[9] The information for this survey was collected from patients with RA disease and the evaluation was based on PRC method. Lee, at al has confirmed that genetic risk factor such as PTPN22 and HLA-DR4 gene inheritance would affect the disease prognosis.[10] Pierer, *et al*. investigated on the prevalence of PTPN22 gene polymorphism among RA patients in Germany by studying on 124 patients and 130 healthy persons. They found that 73% of patients and 25% of the normal subjects had PTPN22 polymorphism.[11] In another study that has been performed on Chinese population, Feng, *et al*. evaluated the prevalence of PTPN22 polymorphism among 200 RA patients and the same number of healthy people. They found 63% frequency in patient comparing to 12% in normal persons.[12] Hashemi, *et al*. evaluated the possible association of PTPN22 rs2476601 and RA in a sample of Iranian population who lived in southeastern regions. Their results showed that PTPN22 rs2476601 CT genotype as well as rs2476601 T allele was a risk factor for susceptibility to RA.[13]

In this study, the prevalence of PTPN22 gene polymorphism was assessed among RA patients and healthy population in Isfahan province.

MATERIALS AND METHODS

This study is a case and control survey that performed in 2012 at Rheumatology Clinic of AlZahra University Hospital, Isfahan, Iran. In this study, 100 patients (case group) and 100 healthy persons (control group) are participated voluntarily. The case group had been selected from RA patients who were living in Isfahan province and visited the clinic for follow out of their treatment. Participants in both groups were age-matched and the ratio of female to male was about 75-25%.

Each volunteers' blood was sampled from one of their peripheral vein and then DNA was extracted through salting out method. The PTPN22 gene R620 W (C1858T) (rs2476601) polymorphism was analyzed using the PCR-restriction fragment length polymorphism method.[7,14,15] Amount of 0.2 μg genomic DNA, 1unit Taq polymerase (Fermentas and Lithuvania), 20 pmol of each primer (upstream primer 5'-TCACCAGCTTCCTCAACCACA-3' and the downstream primer 5'-GATAATGTTGCTTCAACG GAATTT-3'), and 5 nmol deoxyNTPs was used to generate a 220 bp amplicon from the PTPN22 gene. PCR process was performed under the condition that is mentioned in Table 1.

Amount of 10 μg of amplicon were exposed to restriction enzyme digestion with XcmI. Digestion results were visualized on 2.5% agarose gels under ultraviolet light.

The Results of genome analysis were statistically analyzed using Chi-square to find any significant relationship between the polymorphism of interest and disease. In this analysis, significant level was pre-defined at $P \leq 0.05$. To investigate the relationship between age and disease we used of the case and control member's age differences independent *t*-test.

RESULTS

Both case and control group were in a range of 19-81 years old. The average age of the case group was 47.6 ± 15.2 and the control one was 46.9 ± 14.8. The independent T analysis showed that the observed difference between average age of two groups were not statistically significant [Table 2].

Male to female ratio were 22-78% in case group and 25-75% in the control group. The Chi-square analysis demonstrated that this difference is not statistically significant [Table 3].

Table 1: The PCR sub process and performance conditions

PCR sub-process	Duration time	Temperature
Initial denaturation	5 min	95°C
35 cycles denaturation	50 s	94°C
Annealing	50 s	55°C
Extension	50 s	72°C
Final extension	7 min	72°C

PCR: Polymerase chain reaction

Table 3: Sexual distribution of participant in the case and control group

	Case group	Control group	Total
Gender			
Male			
No.	22	25	47
Percent	22	25	23.5
Female			
No.	78	75	153
Percent	78	75	76.15
P value	0.62		

Table 5: Relationship between PTPN22 polymorphism and the age of onset of RA

Average Age	PTPN22 (+)	PTPN22 (−)	*P* value
The average of age of onset	36.7±15.2	41.3±15.8	0.37
The average of age	43.9±13.4	47.6±15.2	0.31

PTPN22: Protein tyrosine phosphates number 22, RA: Rheumatoid arthritis

The genotype analysis showed that only 11 persons in case group and eight persons in control one had PTPN22 gene polymorphism. The statistical Chi-square analysis did not detect significant relationship between this polymorphism and onset of RA ($P = 0.47$) [Table 4]. The odds ratio was 1.42 and corresponding 95% confidence interval was (0.55-3.69).

Another aspect of the disease, which may be influenced by genetic is the age of disease commence. The age of onset of RA in case group was averaged 40.7 ± 15.7, ranging from 5 to 78 years old. The independent *t*-test showed that the observed difference between PTPN22 gene polymorphism and age of onset of RA disease is not statistically significant [Table 5].

Evaluation of frequency of PTPN22 polymorphism in the male and female participants revealed that there is not any considerable discrepancy among men and women [Table 6].

DISCUSSION

A comprehensive study is conducted on the effect of PTPN22 on the onset of RA. Through this study the prevalence of PTPN22 has been investigated among 100 volunteer patients (case group) and 100 healthy people (control group) who live in Isfahan city and suburbs. Gender and age was matched between the case and control groups. According to the genome analysis results, 11 patients in case group

Table 2: Age range of the case and control groups and statistical evaluation

Group	*N*	Average age	SD	Min	Max
Case group	100	47	15.2	19	81
Control group	100	46	14.8	19	81
P value	0.74				

SD: Standard deviation

Table 4: Comparison between case and control group for PTPN22 gene polymorphism

	Case group	Control group	Total
PTPN22			
Positive	11	8	19
Negative	89	92	181
P value	0.47		

PTPN22: Protein tyrosine phosphates number 22

Table 6: PTPN22 polymorphism frequency among male and female participants

Group	Male %	Female %	*P* value
Case	13.6	10.3	0.65
Control	8	8	1
Total	10.6	9.2	0.76

PTPN22: Protein tyrosine phosphates number 22

and 8 patients in the control group has PTPN22 polymorphism. The Chi-square statistical criterion showed that possessing PTPN22 does not have any significant difference between the case and the control group. The time of onset of RA also did not have any relationship with PTPN22 polymorphism. Likewise, the Chi-square statistical test showed no association between polymorphism and patient gender.

These results are the same as Sahin's study finding.[7] on the other hand, Majorczyk's study on Polish population,[6] Pierer's study among German population[11] and Feng's study on Chinese population[12] showed a significant relationship between PTPN22 and RA disease. Song *et al*. have shown that there is an important relationship between PTPN22 Polymorphism heritance and RA disease. Totaro *et al*.[5] have found an association between PTPN22 Polymorphism and Ra disease by studying Italian subjects. Hashemi *et al*.[13] have clarified that PTPN22 Polymorphism is a risk factor for susceptibility to RA disease in Iranian subjects.

Indeed, if there is enough supports for a more extensive study with a larger number of participants, the results might become more reliable. It is very important to note that the obtained different results may also be because of ethnic diversity.

ACKNOWLEDGMENT

It should be mentioned that this work has been supported by Isfahan Medical University as a grant number 390456. We should have many thanks to Ms. Keivani from the genetic laboratory of AlZahra Hospital, for her patiently cooperation.

REFERENCES

1. Kurkó J, Besenyei T, Laki J, Glant TT, Mikecz K, Szekanecz Z. Genetics of rheumatoid arthritis-A comprehensive review. Clin Rev Allergy Immunol 2013; Jan 5, [Epub ahead of print].
2. Malaise O, von Frenckell C, Malaise MG. Genetic and environmental interactions on the development of rheumatoid arthritis. Rev Med Liege 2012;67:305-13.
3. Song GG, Bae SC, Kim JH, Lee YH. The PTPN22 C1858T polymorphism and rheumatoid arthritis: A meta-analysis. Rheumatol Int 2013; [Epub ahead of print]
4. Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J, *et al*. Disorders of the immune system and connective tissue. Harrison's Principles of Internal Medicine. 18th ed. New York: McGraw-Hill; 2012.
5. Totaro MC, Tolusso B, Napolioni V, Faustini F, Canestri S, Mannocci A, *et al*. PTPN22 1858C>T polymorphism distribution in Europe and association with rheumatoid arthritis: Case-control study and meta-analysis. PLoS One 2011;6:e24292.
6. Majorczyk E, Jasek M, Płoski R, Wagner M, Kosior A, Pawlik A, *et al*. Association of PTPN22 single nucleotide polymorphism with rheumatoid arthritis but not with allergic asthma. Eur J Hum Genet 2007;15:1043-8.
7. Sahin N, Gunduz F, Inanc N, Direskeneli H, Saruhan-Direskeneli G. No association of PTPN22 gene polymorphism with rheumatoid arthritis in Turkey. Rheumatol Int 2009;30:81-3.
8. Hinks A, Eyre S, Ke X, Barton A, Martin P, Flynn E, *et al*. Overlap of disease susceptibility loci for rheumatoid arthritis and juvenile idiopathic arthritis. Ann Rheum Dis 2010;69:1049-53.
9. Balsa A, Del Amo J, Blanco F, Caliz R, Silva L, Sanmarti R, *et al*. Prediction of functional impairment and remission in rheumatoid arthritis patients by biochemical variables and genetic polymorphisms. Rheumatology (Oxford) 2010;49:458-66.
10. Lee HS, Korman BD, Le JM, Kastner DL, Remmers EF, Gregersen PK, *et al*. Genetic risk factors for rheumatoid arthritis differ in Caucasian and Korean populations. Arthritis Rheum 2009;60:364-71.
11. Pierer M, Kaltenhäuser S, Arnold S, Wahle M, Baerwald C, Häntzschel H, *et al*. Association of PTPN22 1858 single-nucleotide polymorphism with rheumatoid arthritis in a German cohort: Higher frequency of the risk allele in male compared to female patients. Arthritis Res Ther 2006;8:R75.
12. Feng X, Li YZ, Zhang Y, Bao SM, Tong DW, Zhang SL, *et al*. Association of the PTPN22 gene (-1123G>C) polymorphism with rheumatoid arthritis in Chinese patients. Tissue Antigens 2010;76:297-300.
13. 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. Int J Immunogenet 2013 Jan 27, [Epub ahead of print].
14. Aksoy R, Duman T, Keskin O, Düzgün N. No association of PTPN22 R620W gene polymorphism with rheumatic heart disease and systemic lupus erythematosus. Mol Biol Rep 2011;38:5393-6.
15. Eliopoulos E, Zervou MI, Andreou A, Dimopoulou K, Cosmidis N, Voloudakis G, *et al*. Association of the PTPN22 R620W polymorphism with increased risk for SLE in the genetically homogeneous population of Crete. Lupus 2011;20:501-6.

Source of Support: Isfahan Medical University, **Conflict of Interest:** None declared.