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# Association study of PTPN22 gene polymorphism with Rheumatoid Arthritis in Vindhyan Population

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## Abstract

**R**heumatoid arthritis is a complex disease in which combinations of multiple genetic and non-genetic factors determine susceptibility. It is symmetric, chronic polyarticular arthritis that affects 0.5-1% of the population. PTPN22 encodes for an 807 amino acid residue protein called LYP (lymphoid tyrosine phosphatase), which has been shown to negatively regulate T-cell signaling. A single-nucleotide polymorphism in the PTPN22 gene at nucleotide position 1858 C4T (codon 620), resulting in an arginine-to-tryptophan (CGG to TGG) transition, has been shown to be a gain-of-function mutation, with a more potent negative regulation of T-cell signaling through reduced Lck (leukocyte-specific protein tyrosine kinase)-mediated phosphorylation of the TCRx chain, reduced tyrosine phosphorylation of LAT (linker for activation of T cells), and reduced activation of Erk2. The mutant, LYP-Trp620, has been associated with several autoimmune diseases. PCR is a rapid, inexpensive and simple mean of producing relatively large copy number of DNA molecules from the small amounts of source DNA material, even when the source DNA is of relatively poor quality. The oligonucleotides sequences (primers) were designed to create a recognition site for the restriction enzyme XcmI in the T allele. Overall allele '*C*' was found to be in significantly low frequency in disease group as compared to HC group whereas allele '*T*'was present in significantly high frequency in the disease group ($\chi^2$ = 11.88; d.f. =1; *P*=0.0006)..Carriage rate of allele '*C*' was equivalent to HC group and RA group. Whereas carriage rate of allele '*T*' was high in disease group ($\chi^2$ =s, d.f. = 1; *P*=0.).

**Key- words**: Rheumatoid arthritis, PTPN22, XcmI, PCR.

## Introduction

Rheumatoid arthritis often shortened to RAis an autoimmune, chronic inflammatory disease that causes pain, swelling, stiffness and damage to joints. It primarily involves the joints, but should be considered a syndrome that includes extra articular manifestations, such as rheumatoid nodules, pulmonary involvement or vasculitis, and systemic comorbidities.In general the immune system of human body is consist of many type of cells and proteins to combat againstforeign antigens and infection, when something goes wrong with our immune system, our immune system loss the ability to distinguish our body cells from foreign particlesand antigen, at this point the condition of autoimmunity arise and our immune system start to destroy our body cells and chronic inflammation should be the result (1).

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In addition to inflammation of the synovium, expansion of the synoviocytes leads to thickening of the joint lining and formation of the so-called pannus tissue, which invades and destroys local articular structures and bone. A hyperplastic synovium is the major contributor to cartilage damage in rheumatoid arthritis. Loss of the normally protective effects of synovium (e.g. reduced expression of lubricin).The presence of autoantibodies (seropositivity) is associated with more severe symptoms and joint damage, and increased mortality (3). RA susceptibility, 32 risk factors have been identified. Many researchers believe that a bacterial, viral, or fungal infection may trigger the autoimmune response. Other researchers believe there is a genetic role in the development of rheumatoid arthritis. The severity and chronicity of rheumatoid arthritis varies from person to person. Most people who develop rheumatoid arthritis will do so between the ages of 20 and 60. In general, the earlier those symptoms develop, the more severe the disease will be. RA can also produce diffuse inflammation in the lungs, pericardium, pleura and the sclera of the eye, and also nodular lesions, most common in subcutaneous tissue under the skin. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility (2-5).

RA affects approximately 1% of the population worldwide (Malmström*et.al.* 2014). RA affects between 0.5 and 1% of adults in the developed world



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with between 5 and 50 per 100,000 people newly developing the condition each year (6). Most frequent during middle age and women are affected 2.5 times as frequently as men. The heritability of RA has been estimated to be about 60 % and Rheumatoid arthritis is associated with increased rates of cardiovascular illness (standardized mortality rate, approximately 1.5). The risk of lymphoma is increased among patients with rheumatoid arthritis and is strongly associated with inflammatory disease activity; sustained disease activity confers the highest risk. The etiology of RA is still unclear, and it is considered to be a multifactorial disease with interacting contributions from genetics, infections, and environmental and hormonal factors. Additional knowledge about the genetics of RA can help to identify patients with a prognostically severe and aggressive course of the disease and to promote targeted therapy with potential clinical and economic benefits. Great progress in genetic research of RA has been achieved over the past ten years. In addition to the HLA loci, more than 30 genetic markers are strongly associated with susceptibility to RA (7,3).

Mutations in these candidate genes lead to genetic polymorphism (variation in DNA sequence) among random mating individuals, groups, or populations due to the effects of environment These mutations may be either a single nucleotide polymorphism (SNP) or a variable number of tandem repeats (VNTR) of a short repetitive DNA sequence that may influence the rate of gene transcription, the stability of the messenger RNA (mRNA), or the quantity and activity of resulting protein. Thus, the susceptibility or severity of RA will be influence by possession of specific alleles of polymorphic genes (9). SNPs are the most common type of segregating DNA sequence variations, occurring when a single nucleotide in the genome is altered either by base substitution or gelation whereas VNTR are short nucleotide sequence ranging from 14 to 100 nucleotide long that is organized into clusters of tandem repeats, usually repeated in the range of between 4 and 40 times per occurrence. Each variant is an allele and they are inherited co dominantly. Both SNPs and VNTRs are a resource for mapping complex genetic traits by identifying genes responsible for disease predisposition using association studies. Association studies are population-based genetic studies that examine whether an allele of a certain gene or marker is more often found in individuals with the disease (cases) than in normal (controls) at a significantly higher rate than predicted by hence alone (8-12).

Protein tyrosine phosphate, non-receptor type 22 (Lymphoid tyrosine phosphatase ) is located in chromosome 1 encoded by PTPN22 is an intracellular enzyme associates with the molecular adapter protein CBL and may be involved in regulating CBL function in the T cell receptor signalling pathway that dephosphorylates Src family kinases Lck and Fyn as well as components of the TCR/CD3 complex and other key signaling molecules, so it works as an important negative regulator of T-cell responses.The PTPN22 locus is one of the strongest risk factors outside of the major histocompatability complexthat associates with autoimmune diseases (15). PTPN22 encodes lymphoid protein tyrosine phosphatase (Lyp) which is expressed exclusively in immune cells. A single base change in the coding region of thisgene resulting in an arginine to tryptophan amino acid substitution within a polyproline bindingmotif associates with type 1 diabetes, rheumatoid arthritis, systemic lupus erythematosis, Hashimotosthyroiditis, Graves disease, Addison's disease, Myasthenia Gravis, vitiligo, systemic sclerosis juvenileidiopathic arthritis and psoriatic arthritis.Genetic studies indicate that the association is highly reproducible in populations that carry themutant allele. A transitional mutation changing a cytosine to a thymine at position 1858 in the coding regionof the PTPN22 gene resulted in a single amino acid change ofan arginine to a tryptophan at codon 620 in exon 14. This R620Wamino acid substitution is located in a polyproline motif, PLPXR,within the Lyp phosphatase protein thought to be involved in bindingto SH3 domains during protein–protein interactions (13-19).

Lymphoid tyrosine phosphatase (LYP) encoded by *PTPN22* is an intracellular enzyme that dephosphorylates Src family kinases Lck and Fyn as well as components of the TCR/CD3 complex and other key signaling molecules.Therefore, LYP appears to be an important negative regulator of T-cell responses. PTPN22 encodes for an 807 amino acid residue protein called LYP (lymphoid tyrosine phosphatase), which has been shown to negatively regulate T-cell signaling (21). A single-nucleotide polymorphism in the PTPN22 gene at nucleotide position 1858 C4T (codon 620), resulting in an arginine-to-tryptophan (CGG to TGG) transition, has been shown to be a gain-of-function mutation, with a more potent negative regulation of T-cell signaling through reduced Lck (leukocyte-specific protein tyrosine kinase)-mediated phosphorylation of the TCRx chain, reduced tyrosine phosphorylation of

LAT (linker for activation of T cells), and reduced activation of Erk2. The mutant, LYP-Trp620, has been associated with several autoimmune diseases (20-25).

The presence of the minor 1858T allele increased the relative risk for RA almost two-fold. The carrier frequency of the 1858T risk allele in Caucasians with RA was 28% while this allele was present in approximately 17% of Caucasian controls. PTPN22 has been shown to bind to an intracellular tyrosine kinase, Csk. This binding occurs by virtue of a proline-rich SH3 binding site on *PTPN22*, interacting with the SH3 domain of Csk. As shown in Figure 5, these molecules act in concert to inactivate Lck, aSrc family kinase that is involved in early T cell signaling events. Csk acts to phosphorylate tyrosine 505 (an inhibitory phosphate for Lck), while *PTPN22* acts to remove the activating phosphate at tyrosine 394. The combined effect of these activities is to convert Lck to an inactive configuration (26-30).

The frequency of the 1858T allele was significantly higher in both lepromatousand tuberculoid leprosy patients than in normal healthy controls. Although homozygous 1858TT was absent from both groups of patients, as well as from the control samples studied, heterozygous CT was significantly increased in both groups of patients. All genotype frequencies in patients as well as in controls were in Hardy–Weinberg equi-librium. The allele frequency of the minor T allele varies in different ethnic groups. Contrary to reports of an absence of the T allele in Asia, we observed a very low frequency (1.9%) of this allele in normal healthy individuals from North India as compared with that reported for the populations of European ancestry. Earlier reports of an absence of the 1858T allele in individuals from Asia have been from studies conducted in Japan, Korea, and China; these populations are Mongoloid in origin and differ ethnically from Indians. North Indians have been described as being basically Caucasoids, with a racial admixture of Mongoloid (31-33).

nchial asthma is the most common chronic diseasebamong children in the world. It affects up to 300 million people worldwide. Bronchial asthma is influenced by genetic and environmental factors. The disease is defined by the presence of airway hyper-reactivity, mucus overproduction, and chronic eosinophilic inflammation. Asthma is often characterized by enhanced total serum IgE level upon the exposure to allergens, which is known as an atopy. Many family studies, through genome-wide linkage studies, confirmed the involvement of genetic predisposition in the development of atopy in asthmatic patients. The elevated IgE production in asthmatic patients results in promotion of acute hypersensitivity responses, chronic eosinophil-predominant allergic inflammation with T helper-2 (Th2) cells cytokine production [1].

## Material and Methods

**Patient recruitment:**

Rheumatoid arthritis patients were recruited from Sanjay Gandhi hospital, Rewa, (M.P.) during the year 2016-2017. 112 patients were enrolled in the study. All the patients were of Central Indian origin. The dignosis of RA was based on various laboratory tests (Rheumatoid Factor, Sed rate, Hemocrit, Synovial fluid analysis, Citrulline antibody, Antinuclear antibodies (ANA), C-Reactive Protein(CRP), Anti-CCP antibodies ) and radiological criteria. All patients participating in study provided informed consent. Institutional ethics committee of Shyam Shah medical college, Rewa (M.P.), India, approved the experimental protocol.

**Healthy controls**

125 randomly selected healthy controls (HC) were enrolled in the study. They consisted of medical staff and healthy volunteers from Rewa, Jabalpur, Bhopal, Indore as well as individuals residing in central region of India. Hence, control group was drawn from same area assuming similar environmental and social factors.

**Sample collection**

Approximately 5 ml. of blood sample was collected in 0.5 M EDTA tubes from each RA patients as well as from healthy controls. These samples were stored frozen at -80˚C until DNA was extracted from them.

**DNA isolation**

Genomic DNA was extracted from whole blood by the modification of salting out procedure described by Miller and coworkers (Miller et al. 1988). Frogen blood sample was thawed at room temperature. 0.5 ml. of whole blood sample was suspended in 1.0 ml. of lysis buffer (0.32 M Sucrose, 1 mM Mgcl2, 12 mMTris and 1% Triton-X-100) in a 1.5 ml. microcentrifuge tubes. This mixture was mixed gently by inverting the tube upside down for 1 min. The mixture was then allowed to stand for 10 min. at room temperature to ensure proper lysis of cells. The mixture was centrifuged at 11,000 rpm for 5 min. at 4˚C to pellet the nuclei. The supernatant was discarded carefully in a jar containing disinfectant, as pellet formed is loosely adhered to the bottom of centrifuge tube. The pellet was resuspended in 0.2 ml. of lysis buffer and recentrifuge at 11,000 rpm for 5 min. The pellet was than dissolved in 0.2 ml. of

deionized autoclaved water and mixed thoroughly on vortexer. The mixture was centrifuged at 14,000 rpm for 1 min. at 4˚C. Supernatant was discarded to gain an intact pellet. To the above pellet, 80 µlof proteinase K buffer (0.375 M Nacl, 0.12 M EDTA, pH 8.0) and 10 µl of 10% SDS (10% w/v SDS, pH 7.2) was added. Mixture was well frothed with the help of micro tip to allow proper lysis of pelleted nuclei. After digestion was complete, 100 µlof saturated cold 5M Nacl was added and shaken vigorously for 15 sec. To the above mixture 0.2 ml. of deionized, autoclaved water and 0.4 ml. of phenol-chloroform (4:1 v/v) were added to remove most of the non-nucleic acid organic molecules. Microcentrifuge tube was inverted upside down until the solution turned milky. Phases were separated by centrifuging the above mixture at 12,000 rpm for 10 min. at 4˚C. Aqueous (top) layer was saved and transferred in another microcentrifuge tube. Transfering of any interface layer was avoided. To the aqueous layer, 1 ml. chilled absolute ethanol was added and the tube was inverted several times until the DNA precipitated. DNA precipitates like thread. This was centrifuged at 14,000 rpm for 4 min. at 4˚C to pellet the DNA thread. Supernatant was discarded. The pellet was washed twice with 1 ml. of 70% alcohol. The mixture was again centrifuged at 14,000 rpm for 1 min. 4˚C. Supernatant was discarded and pellet was air dried for 10-20 min. The pelleted DNA was rehydrated in 100-200 µl. of TE buffer pH 7.4 (10 mMTris-HCL pH 7.4, 1mM EDTA, pH 8.0). DNA was allowed to dissolve overnight at 37˚C before quantitating.

**Determination of quality and quantity of isolated DNA**

The isolated DNA is to be used for PCR based study. Therefore its suitability for PCR along with its size heterogeneity is among the most important criterion for purity. As a matter of general practice all DNA preparations were tested for quality and quantity measures, as described in the following paragraphs.

**Quantitation by UV spectrophotometry**

The isolated genomic DNAs were then tested for purity by measuring their absorbance values at 230 nm, 260 nm, 280 nm and 300 nm using a UV visible spectrophotometer (Systronic, India). A DNA preparation was considered to be good if it had A 260 nm / A 280 nm ratio as approximately 1.8 and A 300 nm was 0.1 or lesser. The absorbance at 260 nm was used to calculate the amount of DNA, using the relationship that double stranded DNA at 50µg/ml concentration has an absorbance= 1.0 at 260 nm.

**Agarose Gel Electrophoresis**

Gel electrophoresis of the genomic DNAs was carried out for qualitative estimation of samples prepared. A good DNA preparation appears as single band. A horizontal agarose slab gel electrophoresis apparatus (BangloreGenei, Bangaore, India) was used. In brief, 2 µl of each genomic DNA was loaded on 0.8 agarose (0.8 % w/v, Sigma) containing ethidium bromide solutions (0.5µg/ml) and electrophoresis was done at 80 V in 1x TAE buffer (40 mMTris, 20 mM acetic acid, 1 mM EDTA).

**Polymorphism screening**

In general, the genomic DNA extracted from peripheral blood of healthy individuals and diseased individuals was subjected to PCR followed by restriction digestion and electrophoresis to genotype both the groups for relevant gene of interest.

All the PCRs were carried out in a PTC 200 Thermocycler (MJ Research Inc. USA). PCR is a rapid, inexpensive and simple mean of producing relatively large copy number of DNA molecules from the small amounts of source DNA material, even when the source DNA is of relatively poor quality. Due to the extreme sensitivity, precautions were taken against contamination of the reaction mixture with the trace amounts of DNA, which could serve as an unwanted template. Appropriate negative controlee was included in each PCR run carried out for each gene, to monitor this contamination of PCR mix to avoid any false positive results. The negative controlee used for PCR contained whole PCR reaction mix except target DNA which was replaced by HPLC purified water free of RNase, DNase, and any contamination from any other source resembling the gene sequence.

Subsequently restriction enzyme digestion was performed by incubating the double stranded DNA with appropriate amount of restriction enzyme, in its respective buffer as recommended by the supplier and at optimal temperature for that specific enzyme. A typical digestion includes one unit of enzyme per microgram of starting DNA. One enzyme unit is usually defined as the amount of enzyme needed to completely digest one microgram of double stranded DNA in one hour at the appropriate temperature. Their biochemical activity of the restriction enzyme is the hydrolysis of phosphodiester backbone at specific sites in a DNA sequence. Precaution was taken to avoid star activity of restriction enzymes. When DNA is digested with certain restriction enzymes under non-standard conditions, cleavage can occur at sites different fromthe normal recognition sequence. Such aberrant cutting is called "star

activity" which can be due to high pH (>8.0) or low ionic strength, extremely high concentration of enzyme (>100 U/µg of DNA) and presence of organic solvents in the reaction (e.g. ethanol, DMSO).

The PCR and restriction digestion conditions were optimized for specific locus of relevant segment of the gene to be studied. The PCR products as well as the digested products were separated on either agarose gel or polyacrylamide gel depending on their size. Gels were stained with ethidium bromide solution (0.5 µg/ml) and subsequently visualized and photographed under UV transilluminator.

**Detection of PTPN22 -1858 SNP via PCR-RFLP**

The nucleotide position -1858 (at codon 620) gene has a single nucleotide polymorphism that results in change of nucleotide from cytosine (C) to thiamine (T). The oligonucleotides sequences (primers) were designed to create a recognition site for the restriction enzyme XcmI in the T allele.

**Primer sequences**

Sense-oligo 5'–TCA CCA GCT TCC TCA ACC ACA-3'

Anti-senseoligo 5'-GAT AAT GTT GCT TCA ACG GAA TTTA-3'

**PCR mix**

The PCR reaction was carried out in a total volume of 25 µl containing 100 ng of genomic DNA, 10 pM of each primer, 2mM MgCl2, 0.2 mMdeoxy-nucleotides (dNTPs), 1X buffer and 2U of Taq polymerase.

**Thermal profile**

Thermal profile used for the amplification of desired segment of gene was as follows: Initial denaturation at ….and 35 cycles of denaturation at …., annealing at 60ºC for 1 min and extension at 72 ºC for 1 min, followed by final extention at 74 ºC for 5 min. PCR products were separated on 3% agarose gel using a 100 bp molecular weight (MW) marker to confirm the PCR product size.

**Restriction digestion by *X*cmI**

The C to T transition at codon 620 creates in the 1858T allele a restriction site for *X*cmI. When gene was amplified by PCR than incubated with *X*cmI restriction enzyme (New England Biolabs, USA).

Recognition site for*X*cmI restriction enzyme is:

5'…CCANNNNN^NNNNTGG…3'

3'…GGTNNNN^NNNNNACC…5'

The PCR product was digested using 0.4U XcmI restriction enzyme(New England BioLabs) for 4 h at 371C.

**Genotyping**

Digetion of the amplified 215 bp PCR product gave two fragments of 174 bp and 41 bp respectively if the product was excisable by *X*cmI. Depending on the digestion pattern, individuals were scored as genotype CC when homozygous for presence of *X*cmI site, genotype TT when homozygous for absence of *X*cmI site and genotype CT in case of heterozygosity.

**Statistical Analysis**

The statistical analysis of genotype, allele frequency and carriage rate were done by graph pad prism 3.3 and using Microsoft Excel 2002, Microsoft Corporation.

## Results and Discussion

**Quantitation by UV spectrophotometry;**

The genomic DNA isolated from whole blood of 112 RA patients and 125 healthy controls using salting out method was analyzed spectrophotometrically for yield and purity. All DNA exhibited A260/A280 ratio between 1.5 and 2.0, and had A300 nm values <0.1

**DNA quality assessment by agarose gel electrophoresis and PCR reactions**

Qualitative analysis of isolated DNAs were routinely carried out by visual observation of ethidium bromide stained gels. When genomic DNA was electrophoresed through 0.8% agarose gels, a distinct bright DNA band migrated equivalent to or slower than the 21.1 kb band of Eco RI/ Hind III double digested lambda DNA. This showed that the isolated DNA was of high molecular weight and was also ungraded as inferred from the lack of or minimal smearing seen. Figure.8 shows the gel image having DNA samples of RA patients and HC group.

**Detection of PTPN22- C1855T polymorphism**

Protein tyrosine phosphate, non-receptor type 22 (Lymphoid tyrosine phosphatase) is located in chromosome 1. The 1858C-T (Arg620Trp) single nucleotide polymorphism (rs2476601) was found associated with autoimmune diseases, including rheumatoid arthritis (RA).

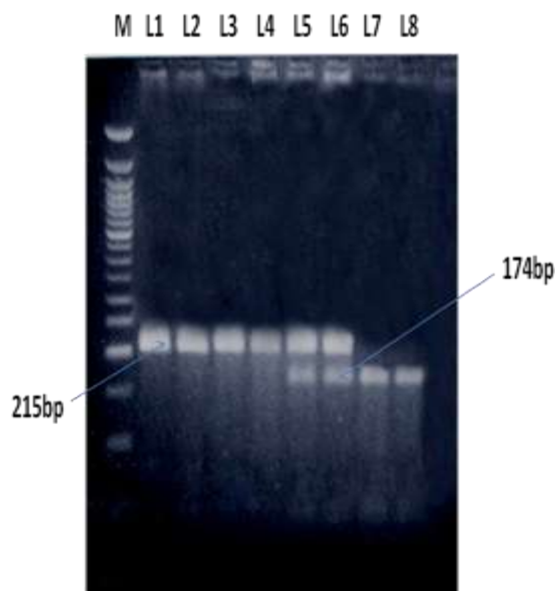



**Fig. 1: Gel picture of Restriction digestion; (L1,2,3,4=C/C, L5,6=C/T and L7,8=T/T).**

Two representative individuals of each of the three genotype C/C(215 bp), C/T(174 bp) or T/T(41 bp). The polymorphism was identified by XCm1 restriction endonuclease digestion of the PCR amplified fragment. Eachdigestion was resolved on 3% agarose gel. Folowing electrophoresis,the gel was stained with ethidium bromide and the fragments were visualized by U.V.

Schematic representation of DNA fragments resolution obtainedupon digestion with Xcm1 enzyme. HC group showed a significant increase in '*CC*' genotype as compared to RA group (78% *vs*55%). In case of genotype'*TT*' was non-significantly distributed in HC group as compared to RA group (2% vs 3%). An odds ratio of 0.34 in RA group respectively for '*CC'*genotype indicated a protective effect of this wild type genotype in our population.

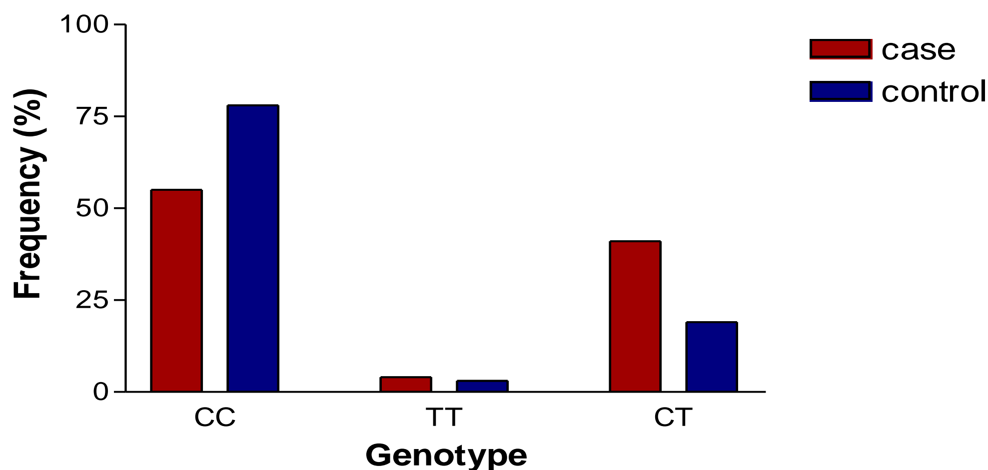
**RFLP-PCR based genotyping assay**.

**Table 5: Genotype frequency, allele frequency and carriage rate distribution of PTPN22-C1858T polymorphism in RA (n= 112) and HC (n= 125) population in Central India.**

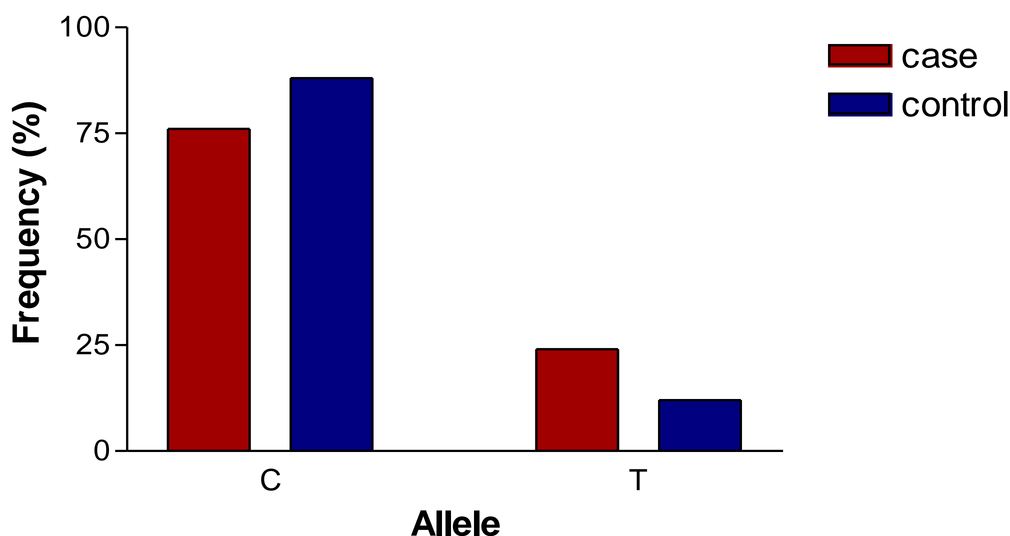
| PTPN22 | Study groups | n | % | $\chi^2$ (*P* Value) | OR | 95% CI |
|---|---|---|---|---|---|---|
| **Genotypes** | | | | | | |
| *CC* | HC | 98 | 78 | | | |
| | RA | 62 | 55 | 14.30(0.0002)* | 0.342 | 0.194 – 0.601 |
| *TT* | HC | 3 | 02 | 0.282(0.594) | 1.543 | 0.329 – 6.88 |
| | RA | 4 | 03 | | | |
| *CT* | HC | 24 | 19 | 13.58(0.0002) * | 2.94 | 1.637 – 5.255 |
| | RA | 46 | 41 | | | |
| --------------- | ------------- | ------------------ | | -------------------- | ------------ | ------------------------- |
| **Alleles** | | | | | | |
| Allele '*C*' | HC | 220 | 88 | 14.49(0.0007)* | 0.43 | 0.263 – 0.700 |
| | RA | 170 | 76 | | | |
| Allele '*T*' | HC | 30 | 12 | 11.88(0.0006)* | | |
| | RA | 54 | 24 | | | |
| --------------- | --------------- | ------------------ | | ---------------------- | ------------ | ------------------------- |
| ***Carriage rates*** | | | | | | |
| Allele '*C*' | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | HC | 122 | 98 | 7.465(0.0063) * | 0.48 | 0.28 – 0.82 |
| | RA | 108 | 96 | | | |
| Allele '*T*' | | | | | | |
| | HC | 27 | 22 | | | |
| | RA | 50 | 45 | | | |

n - Number of individuals carrying particular genotype in a study group. % - Genotype frequency, allele frequency and carriage rates in percentage; *- significant values, $\chi^2$ (*P* Value)- indicates $\chi^2$ *P* Value when HC is compared to RA PTPN22- 1858 Genotype frequencies.




**Graph No1: Genotype frequencies of PTPN22-1858 polymorphism.**




**Graph No2: Allele frequencies of PTPN22-1858 polymorphism.**

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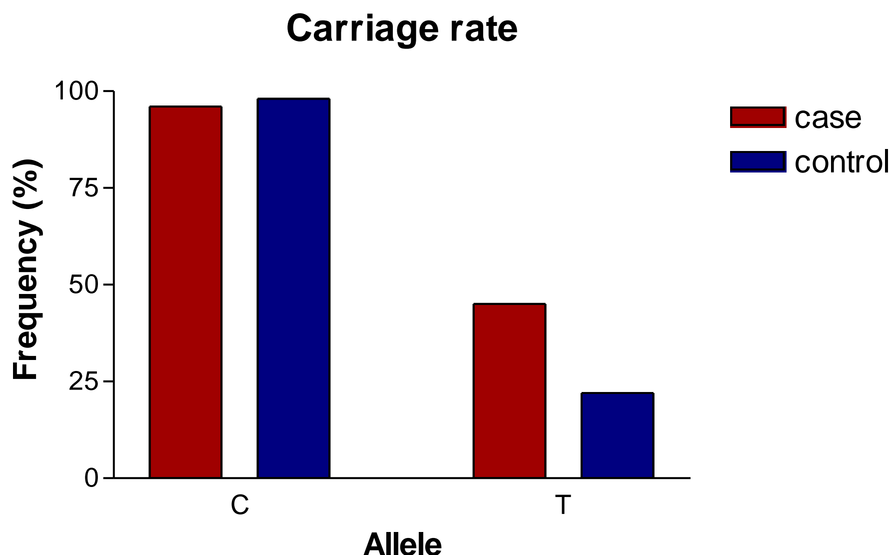


**Graph No3: Carriage rates of allele of PTPN22-1858 polymorphism.**

## Conclusion

DNA sequences of the human genome reveal that many genes are polymorphic. In coding or non-coding regions of a specific gene, there may be either a single base pair substitution of one nucleotide (SNPs) for another or a variable number of repeats of a short (VNTR), repetitive DNA sequence. These variations may influence the rate of gene transcription, the stability of the messenger RNA, or the quantity and activity of the resulting protein. Thus, the susceptibility of severity of a number of disorders will be influenced by possession of specific alleles of polymorphic genes. A single nucleotide polymorphism (SNP) is a site on the DNA in which a single base pair varies from person to person. If a SNP is found within a small, unique segment of DNA, it serves both as a physical landmark and as a genetic marker whose transmission can be followed from parent to child. SNP have gained popularity in recent years and are touted as the genetic markers of choice for the study of complex genetic traits (34).

The idea of using populations of cases and controls for association studies is especially appealing, since such samples are far easier to obtain than the family materials used in conventional linkage analyses. The population based associated studies are based on epidemiologic investigations and designed to compare the frequencies of genetic markers or polymorphic candidate genes between unrelated affected cases and unaffected controls. An association between a disease and a distinct genetic marker (SNP or VNTR) may be suggestive for a causal relationship of an associated gene (i. e., susceptibility gene), or may result for linkage disequilibrium, which may indicate the nearby location of the actual disorder gene (35).

Rheumatoid arthritis (RA) is one of the commonest autoimmune diseases. It is a chronic, progressive, systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis. The exact aetiology of RA remains unknown but is likely to be multifactorial. The pathogenesis of the disease is largely determined by environmental and immunological factors on a genetically predisposed host (36-37).The first signs of joint disease appear in the synovial lining layer, with proliferation of synovial fibroblasts and their attachment to the articular surface at the joint margin. Subsequently, macrophages, T cells and other inflammatory cells are recruited into the joint, where they produce a number of mediators, including the cytokines interleukin-1(IL-1), which contributes to the chronic squeal leading to bone and cartilage destruction, and tumour necrosis factor (TNF-α), which plays a role in inflammation (38-40).

Inter-individual variation in genes encoding proteins with an involvement in the immune and inflammatory responses is a potentially important susceptibility factor in RA as RA is an autoimmune component. Disease association studies on single nucleotide polymorphisms in cytokine genes and cytokine receptor have been extensively studied.

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Moreover up to date, many investigators have tried to explore association between RA and gene polymorphism, of cytokine and cytokine receptor genes reporting positive as well as negative association in different population. But the studies on PTPN22 gene polymorphisms are scarce (41-46).

Our statistical data suggest PTPN22- 1858 polymorphism was significantly associated with rheumatoid arthritis in vindhyan population. HC group showed a significant increase in '*CC*' genotype as compared to RA group (78% *vs*55%). In case of genotype'*TT*' was non-significantly distributed in HC group as compared to RA group (2% vs 3%). An odds ratio of 0.34 in RA group respectively for '*CC'*genotype indicated a protective effect of this wild type genotype in our population. An odds ratio of 1.54 in RA group respectively was consistent with no effect of this genotype in RA susceptibility. The heterozygous genotype '*CT*' was significantly distributed in HC group as compared to RA group (19% *vs 41*%). An odds ratio of 2.94 in RA group showed strong positive association as indicated by highest OR. Overall allele '*C*' was found to be in significantly low frequency in disease group as compared to HC group whereas allele '*T'*was present in significantly high frequency in the disease group ($\chi^2$ = 11.88; d.f. =1; *P*=0.0006)..Carriage rate of allele '*C*' was equivalent to HC group and RA group. Whereas carriage rate of allele '*T*' was high in disease group ($\chi^2$ =s, d.f. = 1; *P*=0.).There are many immunological as well as clinical studies from India describing the pathogenesis of the disease, but till date there no attempts are made to study the genetically aspect of the disease from Central India. This is the first association study from Central India reporting some cytokine genes, PTPN22 gene polymorphisms in RA population as well as healthy control population residing in Central India. Further, both disease group and control population were compared for genotype and allele frequencies of the candidate genes to establish or to search the susceptibility gene for RA that can act as genetic marker for further investigations and therapeutic interventions in future in our population (5). We investigated 112 RA patients and 125 healthy controls for polymorphism in PTPN22 polymorphisms.

Conclusion:

Rheumatoid arthritis is multifactorial caused by interplay of the external and internal environment. Cytokine have an important role in initiation and amplification of inflammatory response in joints in RA and the genes involved in regulation of their activity provide candidate loci for susceptibility to RA. Though there are many immunological and clinical studies in India in Rheumatoid arthritis disease have been reported but to the best of my knowledge there are no reports on the role of PTPN22 in RA patients

We therefore undertook this study to evaluate the possible genetic polymorphism in the PTPN22 genes and to determine whether these polymorphisms can act as genetic marker for susceptibility to Rheumatoid arthritis from Central region of India. To assess this association, we recruited RA patients (n=112) from Sanjay Gandhi hospital, Rewa, Hamidia hospital, Bhopal, Bombay hospital, Indore. Healthy subjects (n=125) of same ethno geographic origin were included in the study. Polymorphisms in PTPN22-1858 gene were detected using PCR-RFLP analysis. Genotype frequencies, allele frequencies and carriage rates were determined for each gene in each study group. Distribution and association of these polymorphisms was analyzed using 3×2 chi square test and fisher's exact test, OR and 95% CI were also calculated. The genotype frequencies of all the study groups included in the study were in accordance with Hardy Weinberg equilibrium. Allele T of PTPN22 was significantly associated with RA susceptibility. These polymorphisms can act as genetic marker for RA susceptibility in RA. However it is required to replicate the data in different populations of different ethno geographic origin and the genetic polymorphism studies should be associated with immunological studies and define these genes as genetic marker.

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