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Association between gene polymorphism of protein tyrosin phosphatase non receptor type 22(PTPN22) and susceptibility for rheumatoid arthritis in an Iraqi population.

العلاقة بين تعدد الاشكال الجيني في جين بروتين تايروسين فوسفيتيز غير المستقبل نوع 22 (PTPN-22) و القابلية لتطوير التهاب المفاصل الرثوي في المرضى العراقيين.

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Abstract:

The gene protein tyrosine phosphatase non-receptor type 22 (*PTPN22*), a negative regulator of T-cell activation and the polymorphism is a single nucleotide polymorphism (SNP) and is a C → T substitution (rs2476601) at nucleotide position 1858 that leads to a tryptophan (W) for arginine(R) transition at codon 620 and this lead to many autoimmune disease like RA. The rheumatoid arthritis patients were diagnosed by physicians of rheumatology in the Rheumatology Center of Al Sadder Medical Teaching Hospital in Al-Najaf Al-Ashraf city. Genomic DNA was extracted from the whole blood samples of patients and controls and by using commercial kit (FavorPrep™ Blood Genomic DNA Extraction Mini Kit). (PTPN22 C1858T SNP) genotyping was done using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method to all participants in this study (60 RA patients and 36 non-autoimmune control group). The genotype and allele frequencies of these SNP were analyzed by statistical tests. There were no significant differences in alleles and genotypes of PTPN22 SNP between RA cases and control group. Patients with PTPN22 CT genotype are more susceptible to infection with rheumatoid arthritis.

Key words: PTPN22, rheumatoid arthritis, single-nucleotide polymorphism, RFLP.

المستخلص:

جين بروتين تايروسين فوسفيتييز غير المستقبل نوع 22 (PTPN22) هو منظم سلبي لخلية تي .ويحصل تعدد الاشكال للجين نتيجه لعده اسباب وهذه الاسباب المؤديه لتعدد الاشكال تؤدي الى تغيير قاعده نايتروجينيه واحده وبالتالي تغير نوع الحامض الاميني من نوع الى اخروبالتالي ذلك التغير ادى الى امراض مناعيه ذاتيه مثل مرض التهاب المفاصل الرثوي و مرض التهاب المفاصل الرثوي شخص من قبل اطباء المفاصل الاختصاص في مستشفى الصدر التعليمي بالتحديد في مركز امراض الروماتيزم في محافظه النجف الاشرف. وباستخدام كت الاستخلاص (FavorPrep™ Blood Genomic DNA Extraction Mini Kit)تم استخلاص الحمض النووي من عينات الدم المرضى والاصحاء. و باستخدام تفاعل سلسلة البلمره(PCR-RFLP)تم معرفه الانواع الجينيه (التنميط الجيني) (PTPN22 C1858T SNP) لكل المشاركين في هذه الدراسة (60 مريض بالتهاب المفاصل الرثوي و 36 مجموعة السيطرة لم يثبت لديهم امراض المناعة الذاتيةولم تكن لديهم اي امراض اخرى). وقد تم تحليل النتائج وتفسيرها ومعرفه التكرارات للانواع الجينيه والاليلات باستخدام الطرق الاحصائيه. ونتيجه البحث لم تكن هناك فروق معنوي احصائي في حاله الاليلات والانواع الجينيه من PTPN22 بين حالات التهاب المفاصل الرثوي ومجموعة السيطرة. واستنتج من البحث ان المرضى اصحاب النمط الجيني CT من C1858T PTPN22 هم الاكثر حساسيه للاصابه بمرض التهاب المفاصل الروماتيزي وايضا استنتج بأن ليس هناك فرق معنوي من ناحيه الجنس الذكور والاناث في تعدد اشكال الجين C1858T PTPN22.

Introduction:

Rheumatoid Arthritis (RA): is a chronic , systemic autoimmune disease that affects joints of the body , and results in warm, swollen, and painful joints, also fever and low energy may be occur . Commonly in the RA wrist and hands joints are involved symmetrically and other parts and organs of the body may be involved, in RA disease the joint capsule become inflamed and thickened, the underlying bone and cartilage are affected and the symptoms of disease come on gradually over weeks to month.[1]

Rheumatoid arthritis is most commonly seen in women more than in men and affects adults between 0.5% and 1% in the developed world and each year there is newly developing the RA condition in a rang between 5 and 50 per 100,000 people.[2]

For autoimmunity disease, the common susceptibility gene that is one of the best examples of a non-associated HLA genes is the C1858T single-nucleotide polymorphism (SNP) of protein tyrosine phosphatase non-receptor type 22 (PTPN22) (rs2476601) [3,4]

the polymorphism of the gene (*PTPN22*) occur in a single nucleotide (single nucleutide polymorphism(SNP)) and is substitution from C to T (rs2476601) at a position of nucleotide (1858) that leads to transition at (620) codon from a tryptophan (W) for arginine(R). [5,6]

The single nucleotide polymorphism in the gene *PTPN22* appears to have a role in a number of auto immune disease not only Rheumatoid Arthritis. this association of this gene in RA and a number of autoimmune disease aiding in rapid progress in the dissection of the pathways of this gene and greater autoimmune diseases understanding[7] .The aims of the study are determination of whether or not single nucleotide polymorphism PTPN22 gene and HLA-DQB1 alleles are associated with susceptibility and the risk of rheumatoid arthritis

-Subjects and methods:

Sixty RA patients including 51 females and 9 males with an age average 44.6± 12.5 and 36 apparently healthy individuals with no history of any autoimmune disease or rheumatic arthritis , Patients with RA and control group were matched in age and sex. The DNA was extracted from blood RA patients and controls using extraction kit (FavorPrep™ Blood Genomic DNA Extraction Mini Kit). The SNP C1858T polymorphism of PTPN22 were determined in RA patients and control group by applying polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Table (1): Genotyping method and primers

SNP	Amplicon	Method		RCR primers sequence	Restriction enzyme	Restriction site
PTPN22	215bp	PCR-RFLP	F	5'-TCACCAGCTTCCTCA ACCACA-3'	XcmI	C/T
			R	5'-GATAATGTTGCTTCA ACGGAATTTA-3'		

SNP single nucleotide polymorphism; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; F forward; R reverse.

-Genotyping

The restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) technique used for PTPN22 gene. PCR was achieved by following conditions:

Initial denaturation	94°C	5min.	1
Denaturation	94°C	30 sec.	35cycle
Annealing	59°C	30 sec.	
Extension	72°C	30 sec.	
Final extension	72°C	5min	1
Hold	4°C	Forever	-

PCR master mix (AccuPower PCR PreMix Kit, Bioneer / Korea) REFLP-PCR

REFLP-PCR Master mix	Volume
PCR product	10µl
XcmI Restriction enzyme buffer 10X	2 µl
XcmI (10 unit)	1 µl
Free nuclease water	7 µl
Total volume	20 µl

Then components of PCR master mix transferred to PCR tube that contain all other components required to PCR reaction depending on standard AccuPower PCR PreMix Kit such as (dNTPs, Taq DNA polymerase, Tris-HCl pH: 9.0, KCl, MgCl2, loading dye, and stabilizer). The PCR product sizes were 215 bp which was digested with restriction enzyme **XcmI**. This restriction enzyme digestion was performed at 37°C overnight.

Anti-cyclic citrullinated peptide (ACCP) antibodies was measured using Aeskulisa kit (Germany), rheumatoid factor (RF) antibodies was detected using Rheumatoid factor latex agglutination (Spinreact / S.A.) and CRP was detected using C-reactive protein latex agglutination (Spinreact / S.A.) .

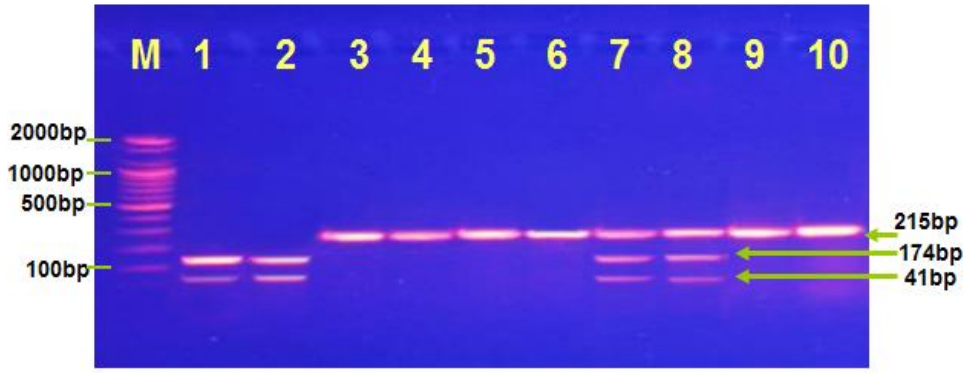

Figure (1): Image of agarose gel electrophoresis that show the product analysis in RFLP-PCR of PTPN22 gene by using XcmI restriction enzyme from some blood patient samples. Where M: marker (2000-100bp), lane (1 and 2) as (CC) homozygote at 174bp and 41bp, lane (3-6 and 9-10) as (TT) homozygote at 215bp and lane (7 and 8) as (C/T) heterozygote at 215bp, 174bp and 41bp.

Results

-Genotype frequency

According to images of Agarose gel electrophoresis that show the genotype distributions of controls and patients as summarized in Table (2), the genotype frequencies of PTPN22 was not significantly difference between the RA patients and control group.

-Allele frequencies

Table (3) showed the allelic frequencies of PTPN22 gene. In this study, the allele frequencies of PTPN22 SNP was not significantly difference between the RA patients and controls group ($P > 0.5$).

Table (2): Distribution of PTPN22 gene polymorphism in RA patients and control group.

ptpn22	Healthy controls (n=36)		Cases with RA (n=60)				
	N	%	N	%	OR	95% CI OR	P
CC	11	30.6	11	18.3	0.51	(0.19 - 1.34)	0.172[NS]
CT	10	27.8	25	41.7	1.86	(0.76 - 4.53)	0.174[NS]
TT	15	41.7	24	40.0	0.93	(0.4 - 2.16)	0.872[NS]

RA rheumatoid arthritis; OR odds ratio; 95% CI=95% confidence interval; P-values for each allele and genotype frequencies are calculated by chi-square test.

Table (3): Distribution of PTPN22 allels in RA patients and control group.

	Healthy controls (n=36)		Cases with RA (n=60)				
Alleles	N	%	N	%	OR	95% CI OR	P
ptpn22-C allele	21	58.3	36	60.0	1.07	(0.46 – 2.48)	0.872[NS]
ptpn22-T allele	25	69.4	49	81.7	1.96	(0.75 – 5.14)	0.172[NS]

-The association between selected positive tests (CRP.RF and ACCP) and PTPN22 genotype and PTPN22 alllels C and T: there is no significant difference between genotype and alleles of PTPN22 with positive tests table 4,5,6 show that:

Table4:The association between selected positive tests and ptpn22 genotype among cases with RA.

	PTPN22 CC(N=11)		PTPN22 CT(N=25)		PTPN22 TT(N=24)		
Positive test	N	%	N	%	N	%	P
CRP	9	81.8	17	68.0	14	58.3	0.39[NS]
RF	10	90.9	18	72.0	20	83.3	0.37[NS]
ACCP	10	90.9	21	84.0	20	83.3	0.83[NS]

Table 5:The association between selected positive tests and ptpn22-T allele among cases with RA.

	ptpn22-T allele negative (n=11)		Ptpn22Tallele positive (n=49)		P value
Positive test	N	%	N	%	
CRP	9	81.8	31	63.3	0.31[NS]
RF	10	90.9	38	77.6	0.43[NS]
ACCP	10	90.9	41	83.7	1[NS]

Table6:The association between selected positive tests and ptpn22-C allele among cases with RA

	ptpn22-C allele negative (n=24)		Ptpn22-C allele positive (n=36)		P value
Positive test	N	%	N	%	
CRP	14	58.3	26	72.2	0.26[NS]
RF	20	83.3	28	77.8	0.75[NS]
ACCP	20	83.3	31	86.1	1[NS]

- **The association between gender and PTPN22 genotype with patients of RA:** this table shows there is no significant association**.**

Table7:The association between gender and ptpn22 genotype among cases with RA.

	PTPN22 CC (N=11)		PTPN22 CT(N=25)		PTPN22 TT(N=24)	
GENDER	N	%	N	%	N	%
Female	8	72.7	21	84.0	22	91.7
Male	3	27.3	4	16.0	2	8.3
Total	11	100.0	25	100.0	24	100.0

Discussion:

In complex autoimmune disease like rheumatoid arthritis that characterized by destruction of the joints that mediated by immune cell and the major contributors to its pathogenesis are genetic factors [8]

The gene of PTPN22 encoding the lymphoid protein tyrosine phosphatase has characterized as a negative regulator of T-cell and B-cell receptor signaling pathways. [9].

Single nucleotide polymorphisms (SNP) in (PTPN22) encoding the lymphoid protein tyrosine phosphatase LYP were linked to numerus autoimmune diseases like RA. [10].

Genotyping of this gene is performed (by technique of restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR)) on all 60 RA patients and 36 controls group.

This current study shows frequency of ptpn22 genotype as follow: ptpn22 CC present in (11/60) (18.3%) in cases vs (11/36)(30.6%) in control ,the OR is (0.51) , P value is (0.172) so this type of gene is protective from RA , the frequency of ptpn22 CT (25/60)(41.7%) in cases vs (10/36)(27.8%) in control ,the OR is (1.86), P is (0.174) so this type of gene is risk for RA while the the frequency of ptpn22 TT (24/60)(40%) in cases vs (15/36)(41.7%) in control ,the OR is (0.93) , P is (0.872).

The results of this study was agreed with other studies that showed there are no significant differences in genotype frequencies between RA patients and controls occurred inTunisian population[11], and the results by study in Iranian population showed C1858T PTPN22 play no role in susceptibility to RA and other autoimmune diseases [12]

There are numerous studies that showed the association among the C1858T PTPN22 SNP and rheumatoid arthritis .[13].

Reason: there is several studies in different European populations showed the allelic heterogeneity distribution has an increasing north-south gradient in the frequencies of alleles of the SNP PTPN22 [14].

This current study show frequency of ptpn22 T allele (49/60) in cases vs (25/36) in control the OR is 1.96 and P is 0.172 while the frequency of ptpn22 C allele (63/60) in cases vs (21/36) in control ,the OR is 1.07 and P is 0.872.

So, these results report no significant difference between ptpn22 alleles frequency and susceptibility of RA.

Meanwhile, several studies have revealed the association between SNP C1858T PTPN22 and RA diseases in Mexican, Spanish, Dutch, Swedish, German, French and British populations. In contrast, some others have showed no any correlation between PTPN22 C1858T polymorphism and RA susceptibility [15]

There are results confirm the dominant variant is +1858T allele that conferring increased risk of disease and significantly documented that +1858T allele is more common in rheumatoid arthritis patients when compared with controls [16]

There were studies that confirm a significant association of the missense SNP PTPN22 as genotype and as allele in UK Population [17].

In multiple populations the genetic architecture of RA is different in European, African, Asian, and Middle Eastern ancestries. [18]

Results by study of this research [19]conclude +1858C/T SNP PTPN22 predisposes peoples to autoimmune disorders because of among thymic development there are enhanced suppression of receptor signaling of T-cell, which allows the survival of T-cells auto-reactive, the significance of PTPN22 in the autoimmune diseases development in south-west of Iran explained by the deficiency in immunological tolerance and the SNP C1858T PTPN22 is an essential genetic risk factor for various autoimmune disorders.

In our study there is no significant association among polymorphism SNP PTPN22 gene and ACCP antibodies, the P value is (0.83).

This study agree with [20] in Khuzestan Province, Southwestern Iran**,** that show there is no association among the SNP +1858C/T PTPN22 gene and the positive sera ACCPs and positive sera RF in RA patients and show no association significantly in the percentage of cases with rheumatoid factor in the TT and CT cases when compare with the CC cases. Other reseach compatible that reported by [21].

While the research by [22] in polish population show weak association of PTPN22 1858 T allele with RF and show strong association with antibodies to cyclic citrullinated peptide (CCP) where(P<0001; OR, 3.2; 95% CI, 2.1-4.9) .

Our results disagree with the following research in northern Sweden that show SNP C1858T of PTPN22 gene in combination with the presence of positive sera anti-CCP antibodies, are associated with the rheumatoid arthritis development. [23]

The discrepancies in the results may simply reflect a clinical heterogeneity across different populations. [24]

A combination of the 1858T variant of the gene PTPN22 and presence of positive sera anti-CCP antibodies gave a much greater relative risk for rheumatoid arthritis developing [25].

The different methods used for detection of RF or the duration time of disease in which the test performed these reason may lead to possible misclassification of rheumatoid arthritis with positive rheumatoid factor cases as negative rheumatoid factor. [26]

A research by [27] reported there is no association of the T PTPN22allele with rheumatoid arthritis in combination with the positive sera rheumatoid factor.

The association of PTPN22 with female and male show no significant difference where P is (0.34),

Our current results disagree with research by [28] in german population that show the male patients suffering from rheumatoid arthritis with high frequency of the 1858T PTPN22 allele, which lead to a greater risk to rheumatoid arthritis development for male compared to female carriers. Spain, Sweden and North America also revealed a significantly stronger effect of SNP C1858T PTPN22 in man compared to women [29].

Possible explanations of the greater effect of SNP PTPN22 in male more than female RA is that environmental influences such as smoking, which are regionally more commonly present in male [30], might contribute to a greater rate of rheumatoid arthritis progress on a background of genetic with a given RA susceptibility and [31] have found an bigger frequency of the 1858T allele PTPN22 in male rheumatoid arthritis patients and suggested that the genetic contribution of this T allele to RA pathogenesis might be more noticeable in men.

In study of [32]in thi-qar show no significant difference in genotypes of PTPN-22 gene between males and females in the genotype T / T, while genotype C/ T showed significant difference between males and females. This result may be due to the subject of her study that mostly females.

Conclusions:

Human with SNP PTPN22 CT genotype are more susceptible to infection with rheumatoid arthritis while the human with PTPN22 CC genotype are protective from RA, there is no significant association between gender of RA patients (female and male) and PTPN22 genotype and there is no significant association between selected positive tests and PTPN22 genotype or allels among RA patients.

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