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Research Article

Association Study of PTPN22 (rs2476601) and PADI4 (rs2240340) Polymorphisms with Rheumatoid Arthritis in Algerian Population

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ABSTRACT

An association between protein tyrosine phosphatase 22 (PTPN22) and Peptidylarginine deiminase 4 (PADI4) genes with rheumatoid arthritis (RA) has been demonstrated in several populations. The present study investigated whether PTPN22 and PADI4 genes polymorphisms were involved in the genetic predisposition to RA in the Algerian patients.

Materials and methods: The PADI4_94 (rs2240340) and the PTPN22 (rs2476601) Single Nucleotide Polymorphisms (SNPs) were genotyped in 300 RA patients and 306 healthy controls by real time polymerase chain reaction method (TaqMan Assays). The relationships between Anti-Citrullinated Peptide Antibody (ACPA) positivity, Rheumatoid Factor (RF) positivity and genotypes were statistically analyzed.

Results: There was no significant association between the PTPN22, PADI4 SNP and RA susceptibility in our population ($p>0.05$). No association with ACPA profile with either PTPN22 or PADI4 was detected ($p>0.05$). However, our results showed a strong association of PTPN22 minor T allele with RF positive disease (OR=8.53 (95% CI 1.34-354.9), p=0.013); also, a significant association was shown between CT genotype of PTPN22 SNP and RF positive RA (OR=8.01 (95% CI 1.22-336.5), p=0.018).

Conclusion: Our findings indicated that PTPN22 and PADI4 polymorphisms were unlikely to play an important role in the susceptibility to RA in Algerian population but PTPN22 polymorphism T allele may predispose individuals to RF positive RA.

Keywords: *PADI4*; *PTPN22*; Rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic inflammatory disease, characterized by chronic synovial inflammation which leads to joint destruction and disability.

A genetic predisposition toward RA has been strongly supported by evidence from twin and sibling studies. Among siblings, the prevalence is 4%. In monozygotic twins, the concordance rate for RA is between 12.3% and 15.4% compared with 3.5% for dizygotic twins [1]. The sibling and twin pair studies demonstrate that genetic factors substantially affect RA susceptibility, resulting in an estimated genetic contribution to RA of approximately 50% to 60% [2,3]. The first genetic risk factor for RA consists of the human leucocyte antigen (HLA) class II molecules. There is extensive evidence showing that certain frequently occurring HLA-DRB1 alleles are associated with susceptibility to RA. The indicated alleles share a conserved amino acid sequence also called the shared epitope (SE) – at position 70 to 74 in the third hypervariable region of the DR β 1 chain [4,5].

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The second genetic risk factor for RA after HLA-DRB1 concerns the C1858T single nucleotide polymorphism (SNP) in the gene encoding for the protein tyrosine phosphatase nonreceptor 22 (*PTPN22*). An association between *PTPN22* and autoimmunity has presently been demonstrated in several cohorts [6,7]. Moreover, some studies have observed that the *PTPN22* T allele confers risk to ACPA-positivity in RA patients [8]. The *PTPN22* gene encodes a lymphoid-specific phosphatase (Lyp) which is an intracellular protein tyrosine phosphatase and an important negative regulator of the TCR signals in lymphocytes activation [9]. The (C1858T, rs2476601) SNP converts an arginine (R) to a tryptophan (W) at position 620 in the PTPN22 protein. It has been suggested that this SNP determines a gain of function of LYP, leading to a stronger suppression of early T cell activation process [10]; unlike other studies in which R620W SNP induced a loss of function of the LYP phosphatase [11].

Another important genetic variant, *PADI4* gene, which is a member of *PADI* family coding for enzymes involved in the post translational conversion of arginine within peptides to citrulline, calcium ion binding and hydrolase activity [12]. A strongest association was observed for a SNP (rs2240340) located in intron 3 of padi4_94 gene and RA susceptibility and anti-citrullinated peptide antibody (ACPA) positivity [13]. The association of this variant with RA susceptibility was observed in different Asian populations [14,15]. However, controversial results were obtained in European Caucasians populations [16-18].

The purpose of this case-control study was to investigate whether *PTPN22* (rs2476601) and *PADI4* (rs2240340) genes polymorphisms are involved in the genetic predisposition to RA and to assess their influence on clinical and immunological features of disease in Algerian RA patients.

MATERIALS AND METHODS

Study population

A total of 300 RA patients (256 women, 44 men) were recruited from the rheumatology department of two centers: Ben Aknoun and Beni Messous teaching hospitals in Algiers, Algeria. All patients met at least four of the seven criteria for the diagnostic classification of RA established by the American College of Rheumatology (ACR 1987). Disease activity was evaluated with disease activity score for 28 joints (DAS28) and functional disability was assessed by the health assessment questionnaire (HAQ). Inflammatory activity was evaluated using erythrocyte sedimentation ration (ESR) and C-reactive protein (CRP). Clinical and biological characteristics of patients are summarized in Table 1.

The control group consisted of 306 matched healthy subjects (sex ratio M/W: 1/4; Mean age 33 ±10 years) with no individual or familial history of autoimmune diseases. Their ages ranged from 19 to 50 years. Informed consent was obtained from all patients and healthy subjects; the study was performed according to the principles laid out in the declaration of Helsinki.

Table 1: Characteristic of patients with RA.

Parameters	RA patients (n=300)
Mean age (years)	48 ± 13
Sex Ratio (Male/Female)	01-Jul
Disease duration (years)	12 ± 8.4
DAS28	4.65 ± 1.41
HAQ	1,33 ± 0,83
RF positive (%)	216 (72%)
ACPA positive (%)	234 (78%)
CRP (mg/l)	5.19 ± 11
ESR (mm/h)	44 ± 30

Abbreviations: ACPA: Anti-Citrullinated Peptide Antibody; CRP: C-reactive protein; DAS 28: Score activity disease of 28 articulations; ESR: Erythrocyte Sedimentation Rate; n: Number of subjects; RF: Rheumatoid Factor.

Note: Values are the mean ± SD (standard deviation), except for RF and ACPA presence which are N (%).

Immunological analysis

The ACPA are measured using the third generation of ACPA-IgG ELISA kits (INOVA Diagnostics, San Diego USA) and titers 20 IU/ml were considered as ACPA positive. The measurement of RF (IgM) was performed using a standard immunonephlometry (BN ProSpec, Siemens) with a cutoff of 15 IU/ml according to manufacturer protocol.

PTPN22 and *PADI4* SNPs analysis

Genomic DNA was extracted from the collected whole blood of RA patients and healthy subjects, using the standard salting out extraction method. All DNA was stored at -20°C until tested. The samples were genotyped for the *PTPN22* rs2476601, and *PADI4* rs2240340 variants (Assay ID: C 16021387_20 and C 609363_20 respectively), by Real-time polymerase chain reation (RT-PCR) using TaqMan technology according to the manufacturer's instructions (Applied Biosystems 7500, Foster City, CA).

Statistical analysis

Comparison of genotypes distribution and alleles frequencies between RA patients and healthy controls was evaluated by the Chi-square (x 2) test, using an odds ratio (ORs) and 95% confidence intervals (95% CIs). Correlation of the associated SNP with autoantibody status among RA cases was performed with x 2 test. Case and control genotypes frequencies did not deviate from Hardy-Weinberg equilibrium. The comparison of the clinical and laboratory parameters with the different genotypes was performed using t-test and x 2 test with Yate's correction when necessary. All statistical analyses were

performed with GraphPad Prism 5 software. A p value lower than 0.05 was considered as statistically significant.

RESULTS

The genotypes and alleles distributions of *PTPN22* and *PADI4* polymorphisms are illustrated in Table 2. The genotype and allele frequencies of the *PTPN22* polymorphism were 93% (CC), 6% (CT), 1% (TT), 96% (allele C) and 4% (allele T) in RA patients, and 90% (CC), 9% (CT), 1% (TT), 97% (allele C) and 3% (allele T) in controls. Concerning *PADI4* variant, the genotype distribution in patients and healthy subjects was 23% vs. 18% for CC, 45% vs. 52% for CT and 32% vs. 28% for TT. For *PADI4* allele distribution, the C allele frequency was 46% in RA patients and 45% in controls. The T allele was present in 54% and 55% of RA and healthy controls respectively. Overall, we did not observe any significant difference in alleles and genotypes frequencies of *PTPN22* and *PADI4* variants between RA patients and healthy controls (p>0.05).

Table 2: Distribution of PTPN22 and PADI4 alleles/genotypes in RA patients and controls.

Variants	RA patient's n (%)	Healthy controls n (%)	OR (95% CI)	p value
PTPN22 (rs2476601)				
CC	279 (93%)	275 (90%)	1.50 (0.81 – 2.81)	0.169
CT	18 (6%)	28 (9%)	0.63 (0.32 – 1.21)	0.143
TT	3 (1%)	3 (1%)	1.02 (1.13 – 7.67)	0.981
C	576 (96%)	594 (97%)	0.73 (0.36 – 1.41)	0.314
T	24 (4%)	18 (3%)	1.38 (0.70 – 2.71)	0.314
PADI4 (rs2240340)				
CC	69 (23%)	54 (18%)	1.39 (0.91 – 2.12)	0.101
CT	135 (45%)	159 (52%)	0.76 (0.54 – 1.05)	0.086
TT	96 (32%)	87 (28%)	1.18 (0.82 – 1.70)	0.339
C	276 (46%)	275 (45%)	1.04 (0.82 – 1.31)	0.71
T	324 (54%)	337 (55%)	0.96 (0.75 – 1.20)	0.71

Alleles and genotypes frequencies in RA patients versus controls were calculated by Chi- square test. p value lower than 0.05 was considered as statistically significant. n: Number of subjects.

Next, we stratified our patients according to the ACPA and RF status (Table 3). Compared to the control group, we did not found any significant difference in genotypes and alleles frequencies of PTPN22 and PADI4 in all subgroups (RF positive and negative, ACPA positive and negative). Also, no significant association was observed between ACPA positive (+) and ACPA negative (-) subgroups for either PTPN22 or PADI4 variants (Table 3). However, the RF positive (+) subgroup had a significantly higher frequency of the minor T allele of PTPN22 when compared to the RF negative (-) group (5% vs. 0.6%; p=0.013; OR=8.53; 95% CI 1.34-354.9) (Table 3). Furthermore, a higher frequency of the CT genotype of PTPN22 SNP was observed in patients with RF (+) as compared with RF (-) patients (8.8% vs. 1.2%; p=0.018; OR=8.01; 95% CI 1.22-336.5). On the contrary, the homozygote genotype CC frequency of this SNP was increased in RF (-) patient group as compared with RF (+) patient group (98.8% vs. 90.7%; p=0.014; OR=0.12, 95% CI 0.00-0.76). Moreover, we explore the possible association of these SNPs with the clinical features of RA (Table 4). Our finding did not demonstrate any significant correlation of the genotypes of PTPN22 and PADI4 with all laboratory (CRP, ESR, ACPA and FR titers) or clinical parameters (disease duration, DAS28 and HAQ scores) of RA patients (p<0.05). In addition, no specific association of these variants to gender was observed in our study (data not shown).

Table 3: Association of PTPN22 and PADI4 variants with RA patients depending on autoantibodies status.

	Genotype frequency n (%)			Allele frequency n (%)		MAF	OR (95% CI)	p value
Variants	**CC**	**CT**	**TT**	**C**	**T**			
PTPN22 (rs2476601)								
Controls (n=306)	275 (90%)	28 (9%)	3 (1%)	594 (97%)	18 (3%)	0.03		
RF+(n=216)	196 (90.7%)	19 (8.8%)	1 (0.5%)	411 (95%)	21 (5%)	0.05	1.69 (0.84-3.40)	0.107
RF-(n=84)	83 (98.8%)	1 (1.2%)	0	167 (99.4%)	1 (0.6%)	0.006	0.20 (0.005-1.27)	0.081
RF+vs. RF-							8.53 (1.34-354.9)	0.013
ACPA+(n=239)	215 (93%)	15 (6,49%)	1 (0,43%)	445 (96,32%)	17 (3,68%)	0.03	1.26 (0.60-2.62)	0.5
ACPA- (n=69)	66 (95,65%)	3 (4,34%)	0	135 (97,83%)	3 (2,17%)	0.02	0.73 (0.13-2.56)	0.622
ACPA+vs. ACPA-							1.72 (0.48-9.28)	0.387
PADI4 (rs2240340)								
Controls (n=306)	54 (18%)	159 (52%)	87 (28%)	275 (45%)	337 (55%)	0.55		
RF+(n=217)	49 (22,6%)	103 (47,5%)	65 (30%)	201 (46,3%)	233 (53,7%)	0.53	1.06 (0.81-1.36)	0.659
RF-(n=83)	18 (21,7%)	33(39,8%)	32 (30,5%)	69 (41,6%)	97 (58,4%)	0.58	0.87 (0.60-1.25)	0.438
RF+vs. RF-							0.82 (0.56- 1.20)	0,296
ACPA+(n=233)	53 (22,7%)	104 (44%)	76 32.6%)	210 (45%)	256 (55%)	0.55	0.99 (0.77-1.27)	0.966
ACPA- (n=67)	14 (20,9%)	32 (47,7%)	21 (31,3%)	60 (44,8%)	74 (55,2%)	0.55	1.01 (0.68-1.49)	0.973
ACPA+vs. ACPA-							0.99 (0.65-1.48)	0,953

Abbreviations: ACPA: Anti-citrullinated peptide antibody; CI: Confidence intervals; N: Number of subjects; OR: Odds ratio; RA: Rheumatoid arthriti; RF: Rheumatoid factor.

Note: Alleles and genotypes frequencies between the different RA phenotypes were calculated by Chi- square test. p value lower than 0.05 was considered as statistically significant.

Table 4: The disease activity and laboratory parameters in relation to PTPN22 and PADI4 genotypes.

Variants	**PTPN22 (rs2476601)**			**PADI4 (rs2240340)**		
	TT	**CT+CC**	**p**	**TT**	**CT+CC**	**p**
Parameters	**Mean ± SD**	**Mean ± SD**				
Disease duration (years)	12,66 ± 0,54	12,61 ± 1,91	0,981	13,93 ± 0,97	12,96 ± 0,62	0,410
DAS28	4,54 ± 0,09	4,15 ± 0,40	0,273	4,55 ± 0,17	4,49 ± 0,11	0,790
HAQ	1,31 ± 0,07	1,77 ± 0,28	0,104	1,24 ± 0,13	1,37 ± 0,08	0,460
CRP (mg/l)	5,61 ± 0,89	6,08 ± 3,70	0,169	1,67 ± 0,29	3,9 ± 0,66	0,062
ESR (mm/h)	45,31 ± 1,83	36,05 ± 6,28	0,889	44,20 ± 5,58	45,35 ± 3,49	0,865
ACPA (IU/ml)	224,9 ± 20,87	157 ± 29,86	0,398	177,2 ± 23,43	226 ± 23,15	0,275

RF (IU/ml)	252,3 ± 39,9	146,5 ± 38,25	0,445	194,1 ± 38,32	239,9 ± 43,95	0,588

Abbreviations: ACPA=anti-cyclic citrullinated peptides; CRP=C-reactive protein; DAS-28=disease activity score; ESR=erythrocyte sedimentation ration; HAQ=health assessment questionnaire; RF=rheumatoid factor; RA=rheumatoid arthritis.

Note: A t-test was used, p>0.05 was considered as not significant.

DISCUSSION

In addition to HLA-DRB1 alleles, several association studies have confirmed the role of other non-HLA genes in susceptibility to RA. Among the confirmed non-HLA loci contributing to RA risk, probably the most relevant associations have been found with *PTPN22* and *PADI4* genes [19-22].

The *PTPN22* gene is the second strongest genetic association with RA, right after HLA-DRB1. The C1858T has been linked to some autoimmune diseases such as type I diabetes, systemic lupus erythematous, Grave's thyroiditis and myasthenia gravis [23-25]. Studies conducted on the functional consequences of this SNP showed conflicting results. It has been suggested that C1858T may lead to a gain of function of Lyp that influences both T cells and B cells activation and probably lead to autoimmunity by compromising tolerance induction [26]. Other studies have demonstrated a loss of function of *PTPN22* gene product in T cells witch can cooperate with hyper responsive B cells to provoke autoantibody production [11,27].

This study was aimed to assess the possible association of *PTPN22* (rs2476601) and *PADI4* (rs2240340) genes polymorphisms and RA susceptibility in a cohort of Algerian patients. No association between these polymorphisms and RA susceptibility was found when comparing genotype and allelic frequencies between RA patients and healthy controls (Table 2). Similar to our results, no association of C1858T SNP has been reported in some southern European populations [28,29], in Spain [28]in Turkish [30] and Iranian [31] populations. However, a significant association of this polymorphism and RA susceptibility was found in several northern European [32-35] and American populations [36]. Also, a recent meta-analysis realized by Nabi et al., has observed that *PTPN22* 1858C/T polymorphism confers a genetic risk factor for rheumatoid arthritis in Caucasian but not in Asian populations [37]. Contrary to our result, the study conducted by Fodil et al., demonstrated a significant association between *PTPN22* (rs2476601) polymorphism and the susceptibility to RA in the Western Algerian population (OR=9.83, (95% CI (4.28-22.56), p=3.32 × 10-11) [38]. Also, this variant showed association with RA ACPA positive and negative phenotype in Egyptians [39]. In Tunisia, the two existing studies regarding this polymorphism show conflicting results [40,41].

A meta-analysis conducted by Totaro et al., have suggested that a North-South gradient seems exist in the distribution of the T1858 allele in both RA patients and controls in Europe and in the Mediterranean area [42]. Indeed, the frequency of the minor T allele appears to be higher in populations of northern Europe (Sweden, Finland, Poland, Great Britain and Germany) as compared to populations from southern Europe and the Mediterranean area (France, Italy, Turkey, Greece, Tunisia) [42].

Our study showed that the frequencies of the minor T allele were 4% in RA patients and 3% in controls. This finding suggests that the frequency of the 1858T variant in our population follow the geographical distribution already described. The association of padi4_94 SNP to RA development was confirmed in different Asian cohorts [13-15]. This genetic effect was much weaker in Caucasian populations. In this study, the padi4_94 polymorphism is not associated with the RA susceptibility (Table 2). Our result is similar to previous British and French studies, and others European cohorts [43,44,14], in Tunisian [45] and Egyptian populations [39].

A recent meta-analysis showed that the risque allele of the PADI4_94 polymorphism is not associated with RA susceptibility in Caucasian population; but, using homozygote contrast, this association was revealed [46]. The most likely explanations for this discordance appear to be the genetic heterogeneity of populations. The stratification of our analysis by the RF status of the RA group demonstrated a significant association between the *PTPN22* T allele and (+) RF group. Similarly, several studies have suggested that the variant 620W would be associated with RF (+) in RA patients [46-49]. Interestingly, all autoimmune diseases reported an association with the *PTPN22* 1858T allele are characterized autoantibodies production [50,51]. These findings support the hypothesis that the *PTPN22* 1858T variant could be involved in both T-cell activity regulation and B-cell auto reactivity.

In our study, the size of patients and controls cohorts could affect the power of genetic association. The replication of this study on a larger cohort is necessary to confirm these results.

The current study has some potential limitations which could contribute to the false positive or negative results. The size of RA patients and controls cohorts could affect the power of genetic association. The replication of this study on a larger cohort is necessary to confirm these results. Also, the lack of association of the studied SNPs does not exclude the implication of the gene in question in the development of RA in our population; more variants of *PTPN22* and *PADI4* genes should be studied to clarify their association with susceptibility to RA.

CONCLUSION

In conclusion, the present study revealed that the *PTPN22* (rs2476601) and PADI4 (rs2240340) polymorphisms were not a risk factor for RA patients from Algeria. However, we observed that the *PTPN22* T allele may be associated with RF positivity. Further studies on larger cohorts are necessary to confirm our findings in the Maghreb African populations.

TRANSPARENCY

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