Association of PTPN22 (rs2476601) and STAT4 (rs7574865) polymorphisms with rheumatoid arthritis in the Western Algerian population

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ABSTRACT

Aim: The aim of the present study was to replicate the association of five risk gene polymorphisms (PTPN22--rs2476601, STAT4-rs7574865, 6q23-rs6927172, IRF5-rs2004640 and TRAF1/C5-rs10818488) with RA in a specific population of the Western Algeria.

Material and methods: The study group comprised 110 patients with RA and 197 ethnically matched healthy control subjects. All polymorphisms were genotyped using predesigned TaqMan® assays. Allele and genotype frequencies in patients and control subjects were compared by chi-square test and odds ratios with 95% confidence intervals. Correction for multiple testing was carried out using the Bonferroni adjustment.

Results: Statistically significant associations with RA were detected. The strongest signal was obtained for PTPN22-rs2476601 with an allelic Pvalue 3.32 x 10^{-11} (OR = 9.83, 95% CI [4.28 – 22.56]). A second significant association was obtained with STAT4-rs7574865 (allelic Pvalue = 4 x 10^{-3}; OR = 1.75, 95% CI [1.16--2.63]). The third SNP, 6q23-rs6927172, showed a significant result of association with RA, but missed our criteria for significance at allelic level after Bonferroni's correction (allelic Pvalue = 0.027; OR = 0.64, 95% CI [0.42 – 0.97]). Finally, IRF5-rs2004640 and TRAF1/C5-rs10818488 showed a significant association only at genotypic level (Pvalues: 3 x 10^{-4} and 2.9 x 10^{-3} respectively) but did not reach statistical significance when comparing allele frequencies (Pvalues: 0.96 and 0.21 respectively).

Conclusions: From this initial study, we can conclude that PTPN22-rs2476601 and STAT4-rs7574865 polymorphisms are clearly associated with the risk of RA in the Western Algerian population.

Keywords: Rheumatoid arthritis; Algerians; PTPN22.

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INTRODUCTION

Rheumatoid arthritis (RA) is a complex autoimmune disease characterized by altered inflammatory and impaired immune responses causing immune-mediated destruction of tissues and organs. Although the aetiology of this autoimmune disease is not completely known, it is nowadays clearly demonstrated that both genetic and environmental factors contribute to the development of RA.

Previous genetic and family studies indicated a significant genetic contribution to RA development[1]. Genetic association of *HLA-DRB1* and RA has been well established in multiple ethnic groups. However, the *HLA* region contributes only 30–50% of genetic component for RA[2].

Advances in genotyping technology have facilitated the application of whole genome association approaches to identify disease causal variants. Recent and several genome-wide and candidate-gene studies reported additional evidence for association of single nucleotide polymorphisms (SNPs) markers in several candidate genes and/or loci such *PTPN22*, *STAT4*, *OLIG3/TNFAIP3 (6q23)*, *IRF5* and *TRAF1/C5* loci with RA[3,4]. All these genes are involved in the immune response and/or its regulation.

A non-synonymous single nucleotide polymorphism (SNP) in the gene encoding *protein tyrosine phosphatase non-receptor 22* (*PTPN22*) is considered as the second risk factor of susceptibility to RA after HLA[5]. The *STAT4* locus has been identified as a confirmed RA susceptibility locus in UK, Korean, Swedish, US, Greek, Co-

lombian, Spanish and US populations[6–11]. A locus, mapped between *OLIG3* and *TNFAIP3* on chromosome 6q was identified in a genome-wide association study (GWAS) of seven common diseases, including RA, carried out by the WTCCC (The Wellcome Trust Case Control Consortium)[4]. Association with this locus *6q23* has been replicated in populations from the UK and USA[12,13]. The recent finding of an association between the *IRF5* gene and systemic lupus erythematosus (SLE)[14], which has been replicated in multiple populations, as well as the association between *IRF5* and RA 15 and inflammatory bowel diseases (IBD)[16], provides additional support for the important role of *IRF5* and the type I Interferon (*IFN*) system in autoimmune diseases. Finally, a GWAS in American and Swedish populations identified a novel locus mapping between *TRAF1* and *C5* loci associated with RA[17]. This association has been replicated in samples from Greece, Netherlands and North American populations[17, 18].

This study was initiated to replicate, in a specific population of the Western Algeria, the association between these five defined genetic markers and RA. To our knowledge, this is the first study to be realized in the Western Algerian population in order to find a genetic association between these polymorphisms and RA.

PATIENTS AND METHODS

Subjects: The study group comprised 110 RA patients from unrelated families living in Oran region (Western Algeria). A control group comprising 197 ethnically matched healthy volunteers have been included in the study (Table I).

All RA patients met the American Rheumatism Association 1987 revised criteria [19] and were recruited from department of Rheumatology of Oran hospital "CHUO" (*Centre Hospitalo-Universitaire d'Oran*). Ethnic bias within the population studied was minimized by excluding patients that were not of Western Algerian origin. Western Algerian origin was defined as having the four grandparents of each individual possessing a Western Algerian ancestry. The control group was recruited on the same basis of ethnic and geographic characteristics. Healthy volunteers were recruited through a local media campaign, followed by selection of individuals who fulfilled our criteria: having a Western Algerian origin, absence of consanguinity in parents and absence of any autoimmune disease for the volunteers or their parents.

Informed consent was obtained from all subjects and the study was conducted according to the declaration of Helsinki Principles, and the ethics committee of *Centre Hospitalo-Universitaire d'Oran (CHUO, Oran, Algeria)* approved the study.

DNA extraction: Peripheral blood was collected into 5 ml tubes containing Ethylenediamine tetraacetic acid (EDTA) and stored at –20ºC until analysis. Genomic DNA was purified from fresh peripheral blood leukocytes by standard DNA extraction methods[20].

Molecular genotyping: Genes and SNPs choice was based on previous published data on RA association from genome-wide and candidate-gene studies[3,4] (Table II). *6q23-rs6927172* was chosen because it was in Linkage Disequilibrium (LD) in the Caucasian population with *6q23-rs6920220*, another SNP in the same genomic region which was confirmed as a risk factor for RA [4,13].

Genotyping of the five gene polymorphisms was carried out with a Taqman® 5' allelic discrimination assay (Table II). It was performed on an ABI-7500 real-time polymerase chain reaction (PCR) machine (Applied Biosystems, now Life Technologies, Foster City,

TABLE I. POPULATION CHARACTERISTICS

Characteristics	RA Cases (%)	Healthy Controls (%)	P value
Number of Subjects	110	197	–
Men	11 (10%)	89 (45.18%)	NS
Women	99 (90%)	108 (54.82%)	NS
Age (years)	48 + 30.5	40 + 22	NS
Rheumatoid Factor positive	31 (32%)	ND	–
Rheumatoid Factor negative	66 (68%)	ND	–

Values are presented as the mean ± SD or number.
RA: Rheumatoid Arthritis; ND: Not Done; NS: Not significant

TABLE II. SNP MARKERS USED IN ANALYSIS

Gene/Locus	db SNP ID	Assay ID	SNP (NCBI Build)	Location	Gene/Function
PTPN22	rs2476601	C__16021387_20	A/G	Chr.1: 114377568	Missense mutation
STAT4	rs7574865	C__29882391_10	G/T	Chr.2:191964633	Intron
6q23 (TNFAIP3/OLIG3)	rs6927172	C___1575580_10	C/G	Chr.6: 138002175	Intergenic
IRF5	rs2004640	C___9491614_10	G/T	Chr.7: 128578301	Intron
TRAF1/C5	rs10818488	C___2783655_10	A/G	Chr.9: 123705087	Intergenic

db: data base ; SNP: single nucleotide polymorphism ; Chr: chromosome ; NCBI: National Center for Biotechnology Information

CA, USA) according to the manufacturer's protocol. Genotyping of each sample was automatically attributed using ABI PRISM® 7500 Sequence Detection System software "SDS" for allelic discrimination.

Quality control: For quality control, positive and negative controls (from CEPH "Centre d'Étude du polymorphisme Humain") were co-genotyped in each genotyping assay and additional 10% of randomly selected duplicates were included. No discrepancy between duplicates was observed in the genotyping data of the five selected SNPs. All control tests had greater than 99% genotype passing rates.

Statistical analyses: Prior to association tests, we checked the Hardy–Weinberg equilibrium in the control group using a standard chi-square test. Results from the control subjects and RA unrelated patients were compared using the chi-square test (2x2 contingency tables) for statistical significance.

The genotype relative risk (GRR) method (a single genotype vs. the others) was used to compare the genotype distribution in controls and patients. The GRR test adjusts the genotype frequencies in the controls to the expected Hardy–Weinberg proportions and yields more accurate risk estimates[21].

P values < 0.05 were considered statistically significant. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated with Woolf's method. Odds ratio was calculated considering the risk allele of each SNP or considering genotypes containing the risk alleles. Correction for multiple testing was carried out using the Bonferroni adjustment. The significance of the p value was assessed at 0.01 (0.05/5 considering 5 SNPs tested).

RESULTS

Demographic and clinical characteristics of all subjects are summarized in Table I. The observed sex ratio of 9 females for 1 male seems to be an ethnic characteristic of the Algerian population as it has been reported in RA cohorts from other Algerian regions (data not published). There is a difference of sex ratio between RA and controls groups but it is not statistically significant.

We analysed five gene polymorphisms (*PTPN22--rs2476601, STAT4-rs7574865, TNFAIP3-rs6927172, IRF5-rs2004640 and TRAF1/C5-rs10818488*) with a prior evidence of association with RA in numerous genome-wide association studies and candidate-gene approaches[3,4]. For all five SNP polymorphisms no deviation from the Hardy-Weinberg equilibrium was observed in the control group (data not shown). We observed a clear significant association between two SNPs (*PTPN22-rs2476601 and STAT4-rs7574865*) and RA in our population, since the other three SNPs (*6q23--rs6927172, IRF5-rs2004640 and TRAF1/C5-.rs10818488*) did not reach statistical significance at allelic level (Table III).

The strongest result was obtained with *PTPN22--rs2476601* with an allelic Pvalue = 3.32×10^{-11} (OR = 9.83, 95% CI [4.28 – 22.56]) (Table III). Moreover, the presence of at least one copy of T-rs2476601 allele was significantly different between RA patients and healthy controls (Pvalue= 8.36×10^{-9}; OR= 12.08 and 95% CI [4.86 - 33.42]).

Another significant association was obtained with *STAT4-rs7574865* (allelic Pvalue = 4×10^{-3} ; OR = 1.75, 95% CI [1.16 – 2.63]). The presence of at least one copy of T-rs7574865 allele showed a significant difference between RA patients and healthy subjects (Pvalue = 8×10^{-4} ; OR = 2.23 and 95% CI [1.35 – 3.71]).

The third SNP, *6q23-rs6927172*, showed a significant association (allelic-Pvalue = 0.027 ; OR = 0.64, 95% CI [0.42 – 0.97]), but missed our criteria for sig-

TABLE III. SNP ASSOCIATION ANALYSIS IN RA-HS CASE-CONTROL SAMPLE

Gene / SNP	Genotype/ /Allele	RA Patients *n=110 (%)*	Controls *n=197 (%)*	X^2	P_{value}	OR (95% CI)
PTPN22 rs2476601	TT	1 (1)	0 (0)			
	TC	33 (30)	7 (4)	45.5	1.31×10^{-10}	
	CC	76 (69)	189 (96)			
	T	35 (16)	7 (2)	43.97	3.32×10^{-11}	9.83 (4.28 – 22.56)
	C	185 (84)	385 (98)			
	TT + TC	34 (31)	7 (4)	45.38	8.36×10^{-9}	12.08 (4.86 – 33.42)
	CC	76 (69)	189 (96)			
STAT4 rs7574865	GG	53 (48)	133 (68)			
	GT	53 (48)	57 (29)	11.65	2.9×10^{-3}	
	TT	4 (4)	7 (4)			
	T	61 (28)	71 (18)	7.88	4×10^{-3}	1.75 (1.16 – 2.63)
	G	159 (72)	323 (82)			
	GT + TT	57 (52)	64 (32)	11.05	8×10^{-4}	2.23 (1.35 – 3.71)
	GG	53 (48)	133 (68)			
6q23 rs6927172	CC	58 (53)	135 (70)			
	CG	49 (45)	50 (26)	11.39	3×10^{-3}	
	GG	3 (2)	9 (4)			
	G	55 (25)	68 (18)	4.86	0.027*	0.64 (0.42 – 0.97)
	C	165 (75)	320 (82)			
	CG + GG	52 (47)	59 (30)	8.61	3×10^{-3}	2.05 (1.26 – 3.32)
	CC	58 (53)	135 (70)			
IRF5 rs2004640	GG	9 (8)	38 (19)			
	GT	80 (73)	97 (50)	15.84	3×10^{-4}	
	TT	21 (19)	60 (31)			
	T	122 (55)	217 (56)	0.002	0.96	1.01 (0.72 – 1.4)
	G	98 (45)	173 (44)			
	GT + TT	101 (92)	157 (81)	6.9	8.6×10^{-3}	2.72 (1.2 – 6.32)
	GG	9 (8)	38 (19)			
TRAF1/C5 rs10818488	AA	7 (6)	38 (19)			
	AG	69 (63)	92 (47)	11.63	2.9×10^{-3}	
	GG	34 (31)	66 (34)			
	A	83 (38)	168 (43)	1.53	0.21	0.81 (0.57 – 1.13)
	G	137 (62)	224 (57)			
	AA + AG	76 (69)	130 (66)	0.24	0.62	1.13 (0.68 – 1.87)
	GG	34 (31)	66 (34)			

*P value not significant after Bonferroni's correction (alpha=0.01)
RA: rheumatoid arthritis; OR: Odds Ratio; SNP: single nucleotide polymorphism

nificance after Bonferroni's correction. However, at the genotypic level, the Pvalue for *6q23-rs6927172* was still significant after Bonferroni's correction (genotypic--Pvalue = 3×10^{-3}) (Table III).

Finally, *IRF5-rs2004640* and *TRAF1/C5-rs10818488* showed a significant association only at genotypic level (P values: 3×10^{-4} and 2.9×10^{-3}, respectively) but did not reach significance when comparing allele frequencies (P values: 0.96 and 0.21, respectively).

DISCUSSION

The aim of the present study was to replicate, among

the specific Western Algerian population, the association of these five risk gene polymorphisms with RA.

A clear significant association was obtained between RA and two SNPs (*PTPN22-rs2476601* and *STAT4--rs7574865*) in our population, since the other three SNPs (*6q23-rs6927172, IRF5-rs2004640* and *TRAF1/ /C5-rs10818488*) did not reach statistical significance (Table III). This preliminary study in the Western Algerian population is in accordance with previous studies reporting the same findings in several populations.

The strongest association with RA was observed with *PTPN22-rs2476601* polymorphism. The first study of the association of *PTPN22-rs2476601* with RA was conducted in 2004[5]. Since, numerous studies confirmed this association in different populations including French[22], UK[23], Finnish[24], Swedish[25], German[26], Dutch[27], Spanish[28] and Canadian[29] populations. However, a study in a Japanese population could not confirm for association as the causal variant was found to have a very low minor allele frequency[30].

Two other studies in Tunisians, a population with a close ethnic origin to Algerians, reported controversial results about the association of *PTPN22-rs2476601* polymorphism with RA. Indeed, Chabchoub *et al.*[31] reported a lack of association while Sfar *et al.*[32] confirmed the association with RA, through two independent case-control studies from distinct regions in Tunisia.

The second most important association was detected with *STAT4-rs7574865* (allelic Pvalue = 4 x 10^{-3}; OR = 1.75, 95% CI [1.16 – 2.63]). Moreover, the presence of at least one copy of T-rs7574865 allele showed a significant difference between RA patients and healthy subjects (Pvalue= 8 x 10^{-4} ; OR = 2.23 and 95% CI [1.35 – 3.71]). Our results are in accordance with all previous studies on association of *STAT4-rs7574865* with RA in different populations[6–11].

6q23-rs6927172 showed a trend of association with RA in our study. However, it did not reach statistical significance after multiple test correction when comparing allelic frequencies. This could be caused by our study design (case-control and the relatively reduced sample size) or represent a true ethnic characteristic of the Western Algerian population. This observation is not very different from a study from another Mediterranean population (Spanish) which reported a weak association between SNPs located in this genomic region and RA[33]. Indeed, Dieguez-Gonzalez *et al.* reported a weak evidence of association both in the *6q23* intergenic region and in the *TNFAIP3* locus. Overall, statistical association was best explained by the interdependent contribution of SNPs from the two loci *TNFAIP3* and the *6q23* intergenic region. Another study in Tunisians reported a trend of association between *rs6920220* and RA[34]. It is now clear that *6q23* locus is a demonstrated candidate for a role in RA. However, our study only provides a suggestive evidence of the involvement of this locus in RA susceptibility.

Concerning *IRF5-rs2004640* a significant association was obtained only at genotypic level (Pvalues = 3 x 10^{-4}) but did not reach statistical significance when comparing allele frequencies (Pvalue = 0.96). A similar result was obtained in the Tunisian population by Maalej *et al.*[35]; in this study, no significant difference was found at the allelic level between RA patients and Healthy controls. However, the genotype TT was more frequent in patients with RA (42.1%) than in the healthy controls (31.4%) (P value = 0.01). Another study reported no RA linkage in 100 French Caucasian trio families; there was no over-transmission of the *IRF5--rs2004640-T* allele from heterozygotic parents to affected patients (P value=0.76)[36]. This could be explained by the fact that the association effect is brought by the homozygote genotype TT independently from the allele frequency.

Similarly to *IRF5-rs2004640, TRAF1/C5-rs10818488* showed a significant association at genotypic level (P values = 2.9 x 10^{-3}) but did not reach statistical significance when comparing allele frequencies (Pvalue = 0.21). Again, this could be explained, by the study design or represent a true ethnic characteristic of the Western Algerian population. Zervou *et al.* and Kurreeman *et al.* [6,18] reported an association between this region and RA in large-scale genetic association studies, while a genome-wide study performed by the Wellcome Trust failed to identify this region as a candidate for RA[4].

Finally, the case–control approach cannot avoid imperfect matching between cases and controls, and the association may be consequently under- or overestimated. The family-based studies are known to be more robust, avoiding this imperfect matching and testing directly Mendel's law by the transmission disequilibrium test.

The current report is the first clear demonstration of non-MHC related susceptibility gene for RA that confers a risk among the Western Algerian population. It is worthwhile to conduct additional population-based studies, including larger groups to confirm these findings and to analyze other genetic factors in the Algerian population.

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