EXTENDED REPORT

Rheumatoid arthritis and genetic markers in Syrian and French populations: different effect of the shared epitope

Leyla Kazkaz, Hubert Marotte, Mayssa Hamwi, Marie Angélique Cazalis, Pascal Roy, Bruno Mougin, Pierre Miossec

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See end of article for authors' affiliations

Correspondence to: Professor P Miossec, Clinical Immunology Unit, Departments of Immunology and Rheumatology, Hôpital Edouard Hérriot, 69437 Lyon Cedex 03, France; miossec@univ-lyon1.fr

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Objective: To investigate whether ethnic differences exist in the effect of the shared epitope and selected cytokine gene polymorphisms on the susceptibility and severity of rheumatoid arthritis in Syria (Damascus) and France (Rhône-Alpes area).

Methods: 156 patients with rheumatoid arthritis and 120 healthy controls from Syria were compared with 512 patients with rheumatoid arthritis and 471 healthy controls from France. Shared epitope status, cytokine gene polymorphisms interleukin (IL)-1B +3954, IL-1RN +2018 and tumour necrosis factor α promoter (−238 and −308) were analysed by enzyme-linked oligosorbent assay. Joint destruction was defined by a right wrist Larsen score ⩾2. Odds ratios (ORs) were calculated.

Results: In both countries, a dose effect was observed between the shared epitope copy number and rheumatoid arthritis (Syria: OR 1 *v* 0 copies = 1.6, p = NS; OR 2 *v* 0 = 15.3, p<0.01; and France: OR 1 *v* 0 = 2.3, p<0.001; OR 2 *v* 0 = 7.2, p<0.001). A dose effect was also observed between the shared epitope copy number and joint destruction in Syria (OR 1 *v* 0 = 2.2, p = NS; OR 2 *v* 0 = 9.9, p<0.01) and France (OR 1 *v* 0 = 1.8, p<0.01; OR 2 *v* 0 = 4.8, p = 0.001). The dose effect of the shared epitope was greater in Syria than in France. Only the −238 tumour necrosis factor α polymorphism was associated with joint destruction in the Syrian population (p<0.05). However, after adjustment for age, sex, disease duration and rheumatoid factor for severity, this association disappeared.

Conclusion: The frequency of the shared epitope was increased in the French population with rheumatoid arthritis and in controls, but the association between the shared epitope and joint destruction was more pronounced in the Syrian population, with an OR of almost 10 for the homozygotes.

Rheumatoid arthritis is a heterogeneous disease of unknown aetiology, where both genetic and environmental factors have important roles in pathogenesis.[1] A genetic contribution to the development of rheumatoid arthritis is estimated to account for about 30% of the disease risk.[2] The strongest association has been reported with human leucocyte antigen (HLA) alleles, in particular with HLA-DRB1 alleles, which share a similar amino acid sequence, called the shared epitope.[3] Many studies on various populations have supported the association of the shared epitope with rheumatoid arthritis severity, but results have been heterogeneous. These differences may be related to ethnic and clinical heterogeneity between populations. Recently, particular rheumatoid arthritis susceptibility alleles (subtypes of DRB1 alleles) have been associated with different ethnic groups (eg, the DRB1*0401 allele in Caucasians and the *0405 allele in Asian populations).[4] It is estimated that about one third to one half of the total genetic contribution of rheumatoid arthritis can be attributed to genes in the HLA complex.[5 6] Other genetic markers of disease severity have been suggested, in particular for cytokines that play a key role in rheumatoid arthritis pathogenesis. Among them, interleukin (IL)-1 and tumour necrosis factor α (TNFα) are central mediators of joint inflammation and destruction in rheumatoid arthritis.[7 8] Different polymorphisms of IL-1B gene (at +3954), IL-1-RN gene (at +2018) and TNFα promoter (at −238 and −308) have been described previously as severity markers of rheumatoid arthritis.[9–11]

A critical issue for these studies is to include a control group, exposed to the same environment. Accordingly, the present study was undertaken to investigate HLA-DRB1 and selected cytokine gene polymorphisms between controls and patients with rheumatoid arthritis in Syria and France. These two countries have major differences not only in genetic background but also in climate, food and parasite exposure. These genetic factors may be responsible for the differences in the clinical course and disease outcome of rheumatoid arthritis observed in these countries.

MATERIALS AND METHODS

Patients

One hundred and fifty six patients with rheumatoid arthritis who met the American College of Rheumatology 1987 criteria for the diagnosis of rheumatoid arthritis[12] and 120 healthy controls from Syria were compared with 512 patients with rheumatoid arthritis and 471 healthy controls from France. French patients were from the Rhône-Alpes region, and Syrian patients from Teshreen Hospital, Damascus. These volunteers, without any known chronic disease, were selected during their annual check-up at a work-related health organisation (Centre ISBA, Lyon, France) or from among personnel of Teshreen Hospital. These rheumatoid arthritis and control populations were included in succession during the same study period (from January 2000 to December 2002) in the respective departments in Syria and France. Clinical evaluation was performed and joint damage evaluated by *x* rays. Clinical parameters of disease activity and joint destruction included age, sex, disease duration and right wrist Larsen score. Laboratory parameters included erythrocyte sedimentation rate and rheumatoid factor. According to the right wrist Larsen *x* ray score, patients were divided into two groups, as described

Abbreviations: HLA, human leucocyte antigen; IL, interleukin; PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism; TNF, tumour necrosis factor

Table 1 Clinical and biological parameters of patients with rheumatoid arthritis in Syria and France

	Healthy populations			Populations with RA		
	Syria (n = 120)	France (n = 471)	p Values	Syria (n = 156)	France (n = 512)	p Values
Age, mean (SD), years	37.58 (12.53)	48.45 (5.16)	NS	44.02 (14.47)	57.56 (14.67)	NS
Sex, % women	49.2	24.8	0.04	80.1	74.8	NS
Disease duration, mean (SD), years	–	–		7.28 (4.87)	10.05 (8.66)	<0.001
Destruction positive, %	–	–		69.9	53.7	<0.001
Right Larsen wrist index, mean (SD)	–	–		2.33 (1.20)	1.99 (1.75)	<0.001
Rheumatoid factor positive, %	–	–		62.9	61.7	NS
Methotrexate treatment, %	–	–		74.0	94.2	NS

NS, not significant; RA, rheumatoid arthritis.

previously:[9 13] destructive arthritis with a Larsen wrist score ⩾2 and non-destructive arthritis with a Larsen wrist score <2 (table 1).

Polymorphism gene typing

After informed consent, blood was collected from patients and controls, and was stored frozen at −20°C until DNA extraction. DNA was extracted using the Phenol Chloroform Protocol or the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany), as recommended by the manufacturer. Genotyping was performed by enzyme-linked oligosorbent assay as described previously.[14] The list of the shared epitope alleles is listed in that publication.

Shared epitope

Exon 2 regions of both HLA-DR and HLA-B were polymerase chain reaction (PCR) amplified using a combination of DR-specific and B-specific primers. DR-specific primer sequences were forward : 5′-CCG GAT CCT TCG TGT CCC CAC AGC ACG-3′; reverse : 5′-TCG CCG CTG CAC TGT GAA G-3′. B-specific primer sequences were forward: 5′-TCG CCG CTG CAC TGT GAA G-3′; reverse: 5′-ATC TCG GAC CCG GAG ACT-3′. The amplification mixture was composed of 50 mM TRIS-HCl, pH 8.8, 15 mM ammonium sulphate, 1.5 mM $MgCl_2$, 50 µM EDTA, 0.01% (w/v) gelatin, 0.2 mM dNTPs, 2.5 U AmpliTaq (Perkin-Elmer, Wellesley, Massachusetts, USA), 0.15 µM HLA-DR primers, 0.3 µM HLA-DR4 primer and 0.4 µM HLA-B primers in a 100 µl reaction volume. 50–200 ng of extracted DNA was used per amplification. Cycling conditions were as follows: 2 min denaturation at 95°C, then 4 cycles with 30 s at 95°C, 30 s at 68°C and 30 s at 72°C, then 4 cycles with 30 s at 95°C, 30 s at 57°C and 30 s at 72°C, then 3 cycles with 30 s at 95°C, 30 s at 64°C and 30 s at 72°C, then 30 cycles with 30 s at 95°C, 30 s at 60°C and 30 s at 72°C, and then 7 min at 72°C. PCR efficiency was checked by agarose gel electrophoresis. Amplicons were hybridised on specific capture probes coated in eight-well strips assembled on a microtitre plate frame, followed by semiautomated washing, colorimetric detection and reading.

Interleukin 1B (+3954)

A single-nucleotide polymorphism (SNP) has been described at position +3954 in exon V.[9 15] The common allele is C and the rare allele is T. Primer sequences and PCR conditions were: forward primer: 5′-TTC AGT TCA TAT GGA CCA GA-3′; reverse primer: 5′-GTT GTC ATC AGA CTT TGA CC-3′; PCR cycles: (95°C, 2 min)×1; (94°C, 30 s)×40; (55°C, 30 s)×40; (68°C, 1 min)×40; and (68°C, 10 min)×1. Amplicons were hybridised on specific capture probes as described for the enzyme-linked oligosorbent assay method.

Interleukin 1-RN (+2018)

This SNP was described in exon 2 at position +2018.[16] The common allele is T and the rare allele is C. Primer sequences and PCR conditions were: forward primer: 5′-GGG CAC ATG GTG GCT GTG CA-3′; reverse primer: 5′-ACC TAG GGT TTG TGC AGG CA-3′; PCR cycles: (95°C, 2 min)×1; (94°C, 30 s)×40; (55°C, 30 s)×40; and (68°C, 1 min)×40; (68°C, 10 min)×1.

Tumour necrosis factor α (−238)

This SNP was described in the promoter region of TNFα gene at position −238. The common allele is G and the rare allele is A. Primer sequences and PCR conditions were: forward primer: 5′-TCA ACG GAC TCA GCT TTC TGA A-3′; reverse primer: 5′-CGG AAA ACT TCC TTG GTG GAG-3′; PCR cycles: (95°C, 2 min)×1; (94°C, 30 s)×40; (55°C, 30 s)×40; (68°C, 1 min)×40; and (68°C, 10 min)×1.

Tumour necrosis factor α (−308)

This SNP was described in the promoter region of TNFα gene at position −308. The common allele is G and the rare allele A. Primer sequences and PCR conditions were: forward primer: 5′-TCA ACG GAC TCA GCT TTC TGA A-3′; reverse primer: 5′-CGG AAA ACT TCC TTG GTG GAG-3′; PCR cycles: (95°C, 2 min)×1; (94°C, 30 s)×40; (55°C, 30 s)×40; (68°C, 1 min)×40; and (68°C, 10 min)×1.

Statistical analysis

The shared epitope and rheumatoid arthritis susceptibility

To analyse the genetic factor association with rheumatoid arthritis susceptibility, different genotypes were compared between patients and controls in each country (table 2). A multivariate logistic model was built to analyse the association between the different genotypes and the disease risk, providing estimations of odds ratio (OR) in comparison with zero.[16] The multiplicative model implies that the OR for two copies of the rare genotype is the square of the OR for one copy of the rare genotype. Likelihood ratio tests were used to compare this model with the full model.[17] Comparison with the full model (equally estimated disease and observed disease risks) was used to test the gene-dose effect. For the comparison of the effect of

Table 2 Distribution of the shared epitope in patients with rheumatoid arthritis and controls in Syria and in France

	Patients	Controls
Syria	**n = 156**	**n = 120**
SE −/−	94 (60.3%)	92 (76.7%)
SE +/−	45 (28.8%)	27 (22.5%)
SE +/+	17 (10.9%)	1 (0.8%)
France	**n = 512**	**n = 471**
SE −/−	191 (37.3%)	290 (61.6%)
SE +/−	227 (44.3%)	161 (34.2%)
SE +/+	94 (18.4%)	20 (4.2%)

shared epitope on rheumatoid arthritis risk between France and Syria, a multivariate logistic model was built including the shared epitope status and the country. A p value <0.05 was considered to indicate statistical significance.

The shared epitope and rheumatoid arthritis severity

To analyse the genetic factor association with rheumatoid arthritis severity, the different genotypes were compared between patients with destructive and those with non-destructive disease. The same multivariate logistic models were constructed. Comparisons were performed as stated above.

The cytokine SNP and rheumatoid arthritis susceptibility and severity

For the four SNPs, the homozygous genotype with the most frequent allele was compared with the two other genotypes combined together. This was performed because of the very low frequency often associated with the rare allele. The same multivariate logistic models were constructed. Comparisons were performed as stated above. All analyses were performed using SPSS v.12.0.

RESULTS

Population characteristics

Table 1 describes the studied populations, which includes 120 controls (mean standard deviation (SD) age 37.6 (12.5) years, women 49.2%) and 156 patients with rheumatoid arthritis (mean (SD) age 44.0 (14.5) years, women 80.1%) from Syria. In France, 471 controls (mean (SD) age 48.4 (5.2) years, women 24.8%) and 512 patients with rheumatoid arthritis (mean (SD) age 57.6 (14.7) years, women 74.8%) were enrolled. The disease duration was higher in the French (10.0 (8.7) years) than in the Syrian (7.3 (4.9) years, p<0.001) population. Patients with rheumatoid arthritis in Syria had more frequent wrist joint destruction (69.9%) than French patients with rheumatoid arthritis (53.7%; p<0.001). The mean right wrist Larsen score was higher in the Syrian group with rheumatoid arthritis (2.3 (1.2)) than in the French group with rheumatoid arthritis (2.0 (1.8), p<0.001). Rheumatoid factor was equally frequent in Syrian and French populations with rheumatoid arthritis (62.9% *v* 61.7%, NS). The number of patients treated with methotrexate was lower in Syria than in France (74.0% *v* 94.2%, respectively; NS).

The shared epitope dose effect on rheumatoid arthritis susceptibility in Syria and France

In Syria, according to the multivariate analysis adjusted by age and sex, the shared epitope was associated with rheumatoid arthritis susceptibility. The shared epitope was highly associated with rheumatoid arthritis risk, whatever the statistical model used. The difference between the full and the multiplicative model was not significant (likelihood ratio = 2.9, p = 0.09). Accordingly, the multiplicative model was selected. By construction, this model implied a high gene–dose effect. Taking zero copies of the shared epitope as a reference, the disease risk was 3.49-fold higher for patients with two copies of the shared epitope (OR = 1.87 (95% CI 1.32 to 9.27)) and 1.87-fold higher for patients with one copy of the shared epitope (OR = 1.87 (95% CI 1.15 to 3.04)) with the multiplicative model. No interactions were noticed either between the shared epitope and age or between the shared epitope and sex.

In France, according to the multivariate analysis adjusted for age and sex, the shared epitope was also associated with rheumatoid arthritis susceptibility. The shared epitope was highly associated with rheumatoid arthritis risk, whatever the statistical model used. The difference between the full and the multiplicative model was not significant (likelihood ratio test 0.6, p = 0.44). Accordingly, the multiplicative model was selected. Taking zero copies of the shared epitope as a reference, the disease risk was 3.49-fold higher for patients with two copies of the shared epitope (OR = 1.87 (95% CI 1.32 to 9.27)) and 2.06-fold higher for patients with one copy of the shared epitope (OR = 2.06 (95% CI 2.02 to 3.36)) with the multiplicative model. No interactions were noticed either between the shared epitope and age or between the shared epitope and sex. When an effect of the shared epitope status on susceptibility between the two countries was evaluated according to the multiplicative model, a significant interaction was observed, with a greater dose effect of the shared epitope in Syria (p<0.001).

Association between the shared epitope and clinical parameters in Syrian and French patients with rheumatoid arthritis

Comparison of Syrian patients with rheumatoid arthritis according to their shared epitope status (table 3) did not show any difference in sex distribution, disease duration, rheumatoid factor positivity or methotrexate treatment. However, patients with rheumatoid arthritis with the shared epitope were significantly older (48.34 (13.36) *v* 41.17 (14.53) years; p = 0.002), had more frequent joint destruction (82.3% *v* 61.7%; p = 0.006) and a higher right wrist Larsen score (2.63 (1.16) *v* 2.13 (1.18); p = 0.01) compared with patients without the shared epitope.

Similarly, no difference was observed in age, sex distribution, and disease duration or methotrexate treatment (table 3) in French patients with rheumatoid arthritis. However, rheumatoid factor positivity was more frequent in patients with rheumatoid arthritis with the shared epitope (69.5 *v* 48.0; p<0.001). As in Syria, shared epitope positive patients with rheumatoid arthritis from France had more frequent joint destruction (63% *v* 38%; p<0.001) and a higher right wrist Larsen score (2.31 (1.70) *v* 1.47 (1.72); p<0.001).

Table 3 Differences between patients with rheumatoid arthritis in Syria and France according to the shared epitope status

	Syria			France		
	SE positive (n = 62)	SE negative (n = 94)	p Values	SE positive (n = 321)	SE negative (n = 191)	p Values
Age (years), mean (SD)	48.34 (13.36)	41.17 (14.53)	0.002	57.99 (14.55)	56.76 (14.90)	NS
Sex, % female	82.3	78.7	NS	74.5	75.4	NS
Disease duration (years), mean (SD)	7.72 (5.23)	6.99 (4.625)	NS	10.23 (8.40)	9.72 (9.15)	NS
Destruction positive, %	82.3	61.7	0.006	63.0	38.0	<0.001
Right Larsen wrist index	2.63 (1.16)	2.13 (1.18)	0.01	2.31 (1.70)	1.47 (1.72)	<0.001
Rheumatoid factor positive, %	70.2	57.3	NS	69.5	48.0	<0.001
Methotrexate treatment, %	73.8	71.4	NS	94.0	94.6	NS

SE, shared epitope.

The shared epitope dose effect on joint destruction in Syria and France

In Syria, according to the multivariate analysis adjusted for age, sex, disease duration and rheumatoid factor status, the shared epitope was found to be associated with rheumatoid arthritis severity. The shared epitope was highly associated with rheumatoid arthritis risk, whatever the statistical model used. The difference between the full and the multiplicative model was not significant (likelihood ratio = 0.09, $p = 0.76$). Accordingly, the multiplicative model was selected. Taking zero copies of the shared epitope as reference, the risk of severe disease was 5.91-fold higher for patients with two copies of the shared epitope (OR = 5.91 (95% CI 1.20 to 29.39)) and 2.44-fold higher for patients with one copy of the shared epitope (OR = 2.44 (95% CI 1.09 to 5.42)) with the multiplicative model. No interaction was observed between the shared epitope and age, sex, disease duration or rheumatoid factor status.

In France, according to the multivariate analysis adjusted for age, sex, disease duration, and rheumatoid factor status, the shared epitope was also associated with rheumatoid arthritis severity. The shared epitope was highly associated with rheumatoid arthritis risk, whatever the statistical model used. The difference between the full and the multiplicative model was not significant (likelihood ratio = 1.0, $p = 0.32$). Accordingly, the multiplicative model was selected. The risk of severe disease was 2.43-fold higher for patients with two copies of the shared epitope (OR = 2.43 (95% CI 1.34 to 4.55)) and 1.56-fold higher for patients with one copy of the shared epitope (OR = 1.56 (95% CI 1.16 to 2.12)) with the multiplicative model. No interaction was observed between the shared epitope and age, sex, disease duration or rheumatoid factor status.

As for the susceptibility, when an effect of the shared epitope status on severity between the two countries was evaluated according to the multiplicative model, a significant interaction was observed, with a greater dose effect of the shared epitope in Syria ($p<0.001$).

IL-1B, IL-1-RN and TNFα cytokine gene polymorphism distribution

The frequencies of the different genotypes for the four SNPs adhered to the Hardy–Weinberg equilibrium. However, a link was observed between the two IL-1B and IL-1-RN SNPs located in the same locus ($\chi^2 = 15.7$, $p<0.005$ in Syria; $\chi^2 = 26.6$, $p<0.001$ in France). For the two SNPs of the TNFα promoter, a link was observed only in France ($\chi^2 = 0.08$, p = NS in Syria; $\chi^2 = 7.4$, $p<0.005$ in France).

No significant differences in the allele frequencies of IL-1B at position +3954, IL-1-RN at position +2018 or TNFα at positions −238 and −308 between controls in Syria and France were noticed. A similar result was observed when comparing patients with rheumatoid arthritis and controls in Syria and France (table 4). A significant difference was observed only for the rare TNFα −308 allele, which was decreased in the Syrian patients with rheumatoid arthritis (5.8% *v* 26.5%; $p<0.001$) when compared with the French patients.

Finally, we investigated the association between cytokine polymorphisms and rheumatoid arthritis susceptibility. The IL-1B, IL-1-RN, and the TNFα −308 polymorphisms were not associated with rheumatoid arthritis. The association between the TNFα −238 polymorphism and rheumatoid arthritis was observed only in the French population (OR = 0.5, 95% CI 0.3 to 0.8, $p<0.05$). However, after adjustment for age and sex, this association disappeared.

We next investigated the association between cytokine polymorphisms and rheumatoid arthritis severity. The IL-1B, IL-1-RN and TNFα −308 polymorphisms were not associated with joint damage in the two countries. The association between the TNFα −238 polymorphism and joint destruction was observed only in the Syrian population (OR = 8.8, 95% CI 1.6 to 49.4, $p<0.05$) in unvariate analysis. After adjustment for age, sex, disease duration and rheumatoid factor status, this association disappeared.

DISCUSSION

Rheumatoid arthritis is a complex polygenic disease, where environmental and genetic factors contribute both to its induction and to this clinical course. The best characterised functional genetic component in rheumatoid arthritis is the association of the HLA human complex alleles, in particular genes encoding HLA-DRB1 molecules.[5 6] The association of shared epitope with rheumatoid arthritis has been extensively reported in Caucasian populations.[18–22] HLA may be a factor in the initiation of the disease, as its prevalence is high or low depending on the frequency of DRB1 alleles carrying the shared epitope in the control population.[23] Previous studies of the association of the shared epitope with rheumatoid arthritis severity have shown inconsistent results. As an example, surveys of patients with rheumatoid arthritis of South Asian origin have shown marked genetic heterogeneity. These studies have been consistent with the shared epitope hypothesis.[24] However, among Indians living in the west of India, rheumatoid arthritis is associated predominantly with the HLA-DRB1*01 or *10 specificities,[25 26] but in the north, it is associated more specifically with HLA-DR4 and the HLA-DRB1*0405 alleles.[27] Among South Asian people living in South Africa, haplotype associations are strongest, with HLA-DR4 among muslims, and with HLA-DR10 among Tamils and Hindus.[28]

The frequency of the shared epitope seems to be higher in patients with chronic rheumatoid arthritis in northern Europe and Caucasians in North America than in Mediterranean countries.[29–31] However, the situation in the control populations is often unclear. To analyse such effects better, we compared the genetic contribution to susceptibility and severity in Syria and France and compared this effect between the countries. In the present study, differences in the shared epitope frequency were observed between the Syrian and French populations. The shared epitope was more frequently observed in the French population with rheumatoid arthritis and in controls compared with the respective Syrian populations. The shared epitope frequency in patients with rheumatoid arthritis in Syria and France was higher than that in the control groups from the same countries. In addition, we observed a gene–dose effect between the shared epitope copy number and rheumatoid arthritis susceptibility in both countries, with a greater effect in Syria. The same dose effect was observed between the shared epitope copy number and joint destruction after adjustment for age, sex, disease duration and rheumatoid factor status. The OR estimate was relatively modest for the association of joint damage, with an effect around 1.5 in France and 2.5 in Syria according to the multiplicative model.

These differences in the association of the shared epitope with joint destruction observed between two ethnic groups may be explained by the different frequencies of specific shared epitope alleles.[32] The specific shared epitope alleles may have different effects according to the ethnic background.[32] For example, in Syria, the frequencies of DRB1*0101, *0404, *0405 and *1001 were higher in patients with rheumatoid arthritis than in the control group (table 5) Moreover, the DRB1*0101, *0404 and *0405 group clearly had a greater association with joint destruction. This result is in line with studies confirming the association between DRB1*0405 and rheumatoid arthritis severity in Asians.[33 34]

Table 4 Cytokine polymorphisms in patients with rheumatoid arthritis and controls in Syria and France

A

	Syria			France		
	Patients (n = 156)	Controls (n = 120)	Odds ratio (95% CI)	Patients (n = 512)	Controls (n = 471)	Odds ratio (95% CI)
IL-1B +3954						
C/C	88 (56.4%)	64 (53.3%)	1	300 (58.6%)	281 (59.7%)	1
C/T or T/T	68 (43.6%)	56 (46.7%)	0.88 (0.6 to 1.4)	212 (41.4%)	190 (40.3%)	1.04 0.8 to 1.3)
IL-1-RN +2018						
T/T	81 (51.9%)	70 (58.3%)	1	292 (57.1%)	251 (53.3%)	1
T/C or C/C	75 (48.1%)	50 (41.7%)	1.3 (0.8 to 2.1)	220 (42.9%)	220 (46.7%)	0.9 (0.7 to 1.1)
TNFα −238						
G/G	138 (88.5%)	109 (90.8%)	1	479 (93.5%)	416 (88.3%)	1
G/A or A/A	18 (11.5%)	11 (9.2%)	1.29 (0.6 to 2.8)	33 (6.5%)	55 (11.7%)	0.5† (0.3 to 0.8)
TNFα −308						
G/G	147 (94.2%)	110 (91.7%)	1	376 (73.5%)	346 (73.6%)	1
G/A or A/A	9 (5.8%)	10 (8.3%)	0.67 (0.3 to 1.7)	136 (26.5%)	125 (26.4%)	1.0 (0.8 1.3)

B

	Syrian patients (n = 156)				French patients (n = 512)			
	With destruction (n = 156)	Without destruction (n = 120)	Odds ratio (95% CI)	χ^2	With destruction (n = 156)	Without destruction (n = 120)	Odds ratio (95% CI)	χ^2
IL-1B +3954								
C/C	62 (39.7%)	26 (16.7%)	1		137 (28.9%)	146 (30.8%)	1	
C/T or T/T	47 (30.1%)	21 (13.5%)	0.9 (0.5 to 1.9)	0.03	99 (20.9%)	92 (19.4%)	1.15 (0.8 to 1.7)	0.53
IL-1-RN +2018								
T/T	25 (16.0%)	22 (14.1%)	1		133 (28.1%)	135 (28.5%)	1	
T/C or C/C	50 (32.1%)	59 (37.8%)	0.7 (0.4 to 1.5)	0.7	103 (21.7%)	103 (21.7%)	1.02 (0.7 to 1.5)	0.01
TNFα −238								
G/G	92 (59.0%)	45 (28.8%)	1		218 (46.0%)	225 (47.5%)	1	
G/A or A/A	17 (10.9%)	2 (1.3%)	4.16 (1.02 to 16.9)	3.9*	18 (3.8%)	13 (2.7%)	1.4 (0.7 to 3.0)	0.9
TNFα −308								
G/G	103 (66.1%)	44 (28.2%)	1		177 (37.3%)	171 (36.1%)	1	
G/A or A/A	6 (3.8%)	3 (1.9%)	0.85 (0.2 to 3.6)	0.05	59 (12.4%)	67 (14.1%)	0.8 (0.6 to 1.3)	0.6

TNF, tumour necrosis factor.
*In unvariate analysis, TNFα −238 single-nucleotide polymorphism was associated with rheumatoid arthritis susceptibility in Syria but not in France. However, this SNP was associated with rheumatoid arthritis severity in France, but not in Syria.

In France, the DRB1*0101, *0404 or *0405 and the DRB1*0401 alleles have been strongly associated with joint destruction. Many studies have shown an association between DRB1*0401 and rheumatoid arthritis severity among northern European Caucasians.[4 32 35] The increased frequency of DRB1*0401 in French patients with rheumatoid arthritis (21.6%) and its lower frequency in Syrian patients with rheumatoid arthritis (2.6%) may explain the significant association with rheumatoid arthritis severity in France, and the absence of this relationship in Syria.

Although the HLA region has been the genomic region most strongly associated with rheumatoid arthritis, it is estimated to explain only 30–40% of the genetic risk,[36 37] emphasising the importance of other genes in disease susceptibility and severity. Moreover, other genes located in close proximity to DRB1 may also be important in rheumatoid arthritis. The TNF gene is in linkage disequilibrium with certain HLA-DRB1 alleles.[38] In our study, after adjustment for age and sex, we failed to show an effect of the TNFα −238 polymorphism on susceptibility to rheumatoid arthritis. We found no significant association between the IL-1B, IL-1-RN and TNFα −308 polymorphisms, and rheumatoid arthritis severity in the two countries. Also, after adjustment for age, sex, disease duration and rheumatoid factor status, we failed to show an effect of the TNFα −238 polymorphism on rheumatoid arthritis severity. This result is concordant with a study on TNFα polymorphisms in a Dutch

Table 5 Subtypes of HLA-DRB1 distribution in controls and patients with rheumatoid arthritis in Syria and France

	Allele	France		Syria	
Controls		**n = 471**		**n = 120**	
	DRB1*0401	41	(8.7%)	2	(1.7%)
	DRB1*0101/0404/0405	134	(28.5%)	24	(20.0%)
	DRB1*1001	7	(1.5%)	2	(1.7%)
Patients with RA		**n = 512**		**n = 156**	
	DRB1*0401	134	(26.2%)	6	(3.8%)
	DRB1*0101/0404/0405	226	(44.1%)	48	(30.8%)
	DRB1*1001	28	(5.5%)	13	(8.3%)

RA, rheumatoid arthritis.

population,[11] although positive association has also been described.[39] In Syria, the dose effect was more important for severity than susceptibility. However, the shared epitope was more frequent in France, again with a dose effect on severity, but with a smaller effect.

In conclusion, an ethnic variation was seen between the French and Syrian populations with rheumatoid arthritis. Such a difference is already explained partly by differences in the control populations. A multitude of factors seem to influence the clinical course of rheumatoid arthritis, including different combinations of genes involved in disease risk. We have shown in this study that the expression of HLA-DRB1 genes is different in Syrian and French patients with rheumatoid arthritis and may contribute to differences in disease progression and outcome.

Authors' affiliations

Leyla Kazkaz, Teshreen Hospital, Damascus, Syria
Hubert Marotte, Mayassa Hamwi, Marie Angélique Cazalis, Bruno Mougin, Pierre Miossec, Hospices Civils de Lyon-bioMérieux Research Unit on Rheumatoid Arthritis, Hopital Edouard Herriot, Lyon, France
Pascal Roy, Service de Biostatistiques HCL, Laboratoire de Biostatistique-Santé, Université Claude Bernard Lyon 1, Lyon, France

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