CONCISE REPORT

IRF5 *rs2004640-T* allele, the new genetic factor for systemic lupus erythematosus, is not associated with rheumatoid arthritis

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Background: Recently, a new genetic factor within the interferon regulatory factor 5 (*IRF5*) gene was demonstrated for systemic lupus erythematosus (SLE) through linkage and association: the *rs2004640-T* allele. *IRF5* is involved in the production of rheumatoid arthritis (RA) cytokines, and SLE already shares with RA one genetic factor within the tyrosine phosphatase *PTPN22* gene.

Aim: To test the hypothesis that the SLE *IRF5* genetic factor could also be shared with RA.

Patients and methods: 100 French Caucasian trio families with RA were genotyped and analysed with the transmission disequilibrium test, the frequency comparison of the transmitted and untransmitted alleles, and the genotype relative risk. 97% power was available to detect at least a trend in favour of a factor similar to that reported for SLE.

Results: The analysis showed the absence of linkage and association globally and in ''autoimmune'' RA subsets, with a weak non-significant trend against the *IRF5 rs20046470-T* allele. Given the robustness of familial-based analysis, this slight negative trend provided strong evidence against even a weaker factor than that reported for SLE.

Conclusion: Our results exclude the *IRF5 rs2004640-T* allele as a major genetic factor for RA in this French Caucasian population.

Interferon regulatory factor 5 (*IRF5*) is involved in the production of cytokines implicated in the pathophysiology of rheumatoid arthritis (RA), such as tumour necrosis factor α, interleukine 6 and type I interferon.[1]

Recently, the haplotype bearing the *IRF5 rs2004640-T* allele (XTXT, table 1[2]), which confers increased expression and unique splicing variants, was convincingly shown to be a new genetic factor for systemic lupus erythematosus (SLE).[2 3] Linkage was shown by an overtransmission of the *rs2004640-T* allele from heterozygotic parents to patients with SLE ($p<0.001$).[3] The replicated association showed an allele frequency increasing from 0.51 in the general population to 0.61 in the patient population, with a risk conferred by the homozygotic *T/T* genotype greater than that of the heterozygotic genotype ($p<0.001$).[2]

SLE and RA, which show some degree of familial aggregation,[4] have recently been found to share one genetic factor, the *PTPN22-1858T* allele, also shared by several other autoimmune diseases.[5–8] Estimated from the overtransmission observed in our trio sample with RA, 61%, the estimated allele sharing in affected siblings would be 52%,[6] which is virtually undetectable with the available sample.[9]

Altogether, these results prompted us to put forth the hypothesis that this SLE *IRF5* factor could also be associated with RA. The absence of linkage suggestion in genome scans at its location 7q32 was not sufficient, as shown by *PTPN22*, to exclude its being an RA gene.[10 11] Our aim was to test this hypothesis through linkage and association.

PATIENTS AND METHODS

Study design and study population

Patients with RA and family members were recruited through a national media campaign in France, followed by the selection of individuals fulfilling the American College of Rheumatology 1987 criteria for RA,[12] according to the rheumatologist in charge of the patient.[13] All clinical data were reviewed by rheumatologists of our team. Families with an additional affected sibling and patients with RA aged <18 years were excluded. All individuals provided informed consent, and the ethics committee of the Hôpital Bicêtre, France, approved the study.

A set of 100 trio families (one patient with RA and both parents) of French Caucasian origin, as checked for the four grandparents, was studied. Among the 100 patients with RA, 87 were women; mean (SD) age at disease onset was 32 (10) years; 90 presented erosions; 81 were rheumatoid factor positive (RF+); 3 had another autoimmune disease; and 30 had at least a relative, of which 20 were parents, with RA (12 (6%) parents) or another autoimmune disease (AID+; 8 (4%) parents); 78 carried at least one *HLA-DRB1* shared epitope allele and 32 carried at least one *PTPN22-1858T* allele.[6]

Molecular genotyping method

Genomic DNA was purified from fresh peripheral blood leucocytes by standard methods.

Genotyping of the *IRF5 rs2004640* polymorphism was carried out with a Taqman 5′ allelic discrimination assay on an ABI 7500 real-time PCR machine (assay: C___9491614_10). CEPH (Centre d'Etude du Polymorphisme Humain) controls (1347–02 and 884–15) were co-genotyped with all our samples for quality control.

STATISTICAL ANALYSES

Hardy–Weinberg equilibrium

The Hardy–Weinberg equilibrium was checked in the control group (constituted by the untransmitted parental chromosomes) and in the parents before the analysis.

Abbreviations: AID, autoimmune disease; *IRF5*, interferon regulatory factor 5; RA, rheumatoid arthritis; RF, rheumatoid factor; SLE, systemic lupus erythematosus

Table 1 Absence of rheumatoid arthritis association with the interferon regulatory factor 5 *rs2004640-T* allele and the *T/T* homozygotic genotype

Sample	*rs2004640-T* allele frequency		AFBAC	Genotype						GRR	
				Cases (frequency)			Controls (frequency)				
	Cases	Controls*	OR (95 % CI)/p value	*G/G*	*G/T*	*T/T*	*G/G*	*G/T*	*T/T*	OR (95 % CI)/p value	OR (95 % CI)/p value
										T/T vs T/G+G/G	*T/T vs G/G*
Global set (n = 95)	0.54	0.56	0.94 (0.63 to 1.41)/0.76	18 (0.19)	51 (0.54)	26 (0.27)	19 (0.20)	47 (0.495)	29 (0.305)	0.86 (0.46 to 1.61)/0.63	0.95 (0.41 to 2.19)/0.9
RF+ (n = 76)	0.55	0.56	0.95 (0.61 to 1.49)/0.82	14 (0.18)	40 (0.53)	22 (0.29)	13 (0.17)	41 (0.54)	22 (0.29)	1 (0.5 to 2.02)/0.76	0.93 (0.46 to 2.43)/0.88
AID+ (n = 31)	0.55	0.58	0.88 (0.43 to 1.79)/0.72	6 (0.19)	16 (0.52)	9 (0.29)	5 (0.16)	16 (0.52)	10 (0.32)	0.86 (0.29 to 2.53)/0.71	0.75 (0.17 to 3.33)/0.71
PTPN22-1858T+ (n = 32)	0.50	0.61	0.64 (0.32 to 1.29)/0.22	6 (0.19)	20 (0.62)	6 (0.19)	4 (0.13)	17 (0.53)	11 (0.34)	0.44 (0.14 to 1.39)/0.1	0.36 (0.07 to 1.8)/0.20

AFBAC, affected family-based controls; AID+, history of autoimmune disease; GRR, genotype relative risk; *IRF5*, interferon regulatory factor 5; RA, rheumatoid arthritis; RF+, rheumatoid factor positive.
Samples are as in table 2; AFBAC compares the percentage of transmitted and untransmitted *rs2004640-T* alleles with patients, and GRR compares the genotypic repartition between cases and controls.*
*Controls are "virtual controls" derived from untransmitted alleles for each trio family.

Linkage and association analysis

Linkage and association analysis relied on the transmission disequilibrium test for the linkage and on the comparison of allelic frequencies (AFBAC) and the genotype relative risk for the association. These tests have already been described in Dieude *et al*.[6] Given the hypothesis of a shared autoimmune factor, we planned a priori pertinent subgroup analyses: families whose index case had the most common RA autoantibody in the serum—that is, RF+, families with a history of autoimmune diseases (AID+) and families whose index carried the autoimmunity genetic factor *PTPN22-1858T*. Significance was considered for $p<0.05$.

Power calculation

Following the hypothesis of an RA association profile of *IRF5* similar to that observed in SLE, we used the reported allelic frequencies of 61% in patients and 51% in controls.[2] Using the binomial distribution, we had a 97% power to detect a trend in favour of an association: probability of having the frequency in patients superior to that in controls following the binomial distribution for n observations (0–200 in our trio index). The power to detect a significant ($p<0.05$) association was estimated via an Arcsinus transformation[14] creating a variable $Y = \text{Arcsinus}\sqrt{P}$ to replace the frequency P. Its distribution tends to be normal and its variance is thus constant. We estimated $\Phi = |\text{Arcsinus}\sqrt{p1} - \text{Arcsinus}p2|$, which is tabulated on α and power. Using the patient and control frequencies on 200 alleles samples, we obtained $\Phi = 2.02$, corresponding to a power of 64% for $\alpha = 5\%$.

RESULTS

The 295 *IRF5 rs2004640* genotypes obtained for the 100 French Caucasian trio families with RA showed no significant deviation from the Hardy–Weinberg equilibrium in controls, using either the two untransmitted chromosomes of each trio family as one virtual control or the parents. The genotype data were made available to the scientific community at http://www.genhotel.com (at the date of publication).

No RA linkage was observed: there was no overtransmission of the *IRF5 rs2004640-T* allele from heterozygotic parents, but instead a slight (non-significant) undertransmission: (T = 49%, p = 0.76; table 2). This undertransmission was also observed in the pertinent subgroups planned a priori: RF+, AID+ and *PTPN22-1858T*+ families (table 2).

There was no association with RA either. The *rs2004640-T* allele frequency was slightly lower (not significantly) in patients with RA than in the virtual controls derived from untransmitted parental chromosomes and thus being exempt of any stratification bias: 0.54 vs 0.56, p = 0.76 (table 1). This result was also observed for the pertinent subgroups (table 1). There was a slight non-significant decrease of the *rs2004640-T/T* genotype in patients with RA compared with controls in the global sample and pertinent subgroups (table 1).

DISCUSSION

Our aim was to test the *IRF5 rs2004640-T* allele for linkage to, and association with, RA. Our results show a clear absence of RA linkage (default of transmission T = 49%, <50%) and association (*rs2004640-T* allele and *T/T* genotype frequencies lower in patients than in controls: 0.54 vs 0.56 and 0.274 vs 0.305, respectively) in the Caucasian French population investigated. Similar results were observed in the autoimmune subsets of families with RA, AID+ and *PTPN22*+.

The results were obtained with a particularly robust method, the family-based analysis, permitting (1) the direct test of the universal Mendel's law and (2) avoiding the inevitable imperfect population match between patients and controls.

Table 2 Absence of rheumatoid arthritis linkage to the *IRF5 rs2004640-T* allele using the transmission disequilibrium test

Sample	Transmitted	Untransmitted	T (%)	p Value*
Global set (n = 95)	43	45	49	0.76
RF+ (n = 76)	32	33	49	0.90
AID+ (n = 31)	14	16	47	0.71
PTPN22-1858T+ (n = 32)	12	19	39	0.21

AID, autoimmune disease; RF, rheumatoid factor.
AID+, subgroup of families with at least one member (up to the second-degree relatives) with an AID; n, number of genotyped trio in each sample; *PTPN22-1858T*+, subgroup of families with index RA case carrying the *PTPN22-1858T* allele in the genotype; RF+, subgroup of families with index rheumatoid arthritis (RA) case positive for RF; T, percentage of transmission of the *rs2004640-T* allele from heterozygotic parents to RA cases, compared with Mendel's expectation of 50%.
*Using the χ^2 test with 1 degree of freedom.

Those results allow us to exclude this allele as a significant genetic factor for RA, globally or in autoimmune RA subsets, for this population. Indeed, we had a 97% power to detect at least a trend in favour of a factor similar to the reported SLE genetic factor. As we did not observe even the slightest trend, in RA or in any of the autoimmune subsets, there is no evidence to suggest that it could be a more modest factor, requiring a larger sample size to be detected. In addition, our results provide an accurate estimate of the *rs2004640-T* allele frequency in the French Caucasian population, fulfilling the criteria of being from French Caucasian decent for each of the four grandparents. This observed frequency, 0.55 in mean, was similar to that observed in reported control Caucasian populations (ranging from 0.44 to 0.56).[2 3]

To our knowledge, this is the first report excluding an involvement of this new autoimmune disease genetic factor in RA, using both linkage and association in a Caucasian population.

These results cannot be extrapolated to other populations. Given the relatively early onset of RA in this family sample, additional studies in the general RA population would be useful to exclude *IRF5* as a minor factor in RA. Other populations need to be investigated to define how general this absence of involvement in the genetics of RA could be. Like *PTPN22*, involved in many autoimmune diseases but not all,[15] *IRF5* could still be implicated in autoimmune diseases other than SLE, if not in RA.

There is now convincing evidence to support the hypothesis of a shared genetic background for subsets of autoimmune diseases. The recent findings concerning *PTPN22* confirm this hypothesis.[6–8] It will be particularly interesting to determine whether *IRF5* is a specific SLE factor or a more general autoimmune disease factor, through the investigation of other diseases, in particular those associated with SLE, such as type 1 diabetes and autoimmune thyroid diseases.[4]

In conclusion, we provide strong and robust evidence against the involvement of the *IRF5 rs2004640-T* allele, the new SLE genetic factor, in the genetics of RA, at least in the French Caucasian population. It remains to be determined whether this factor is SLE specific or shared with other autoimmune diseases.

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