CONCISE REPORT

The *TNFRSF1A* R92Q mutation is frequent in rheumatoid arthritis but shows no evidence for association or linkage with the disease

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Objective: *TNFRSF1A* mutations cause *TNFRSF1A*-associated periodic syndrome (MIM#142680). A recent study suggested that the R92Q mutation was associated with polyarthritis. We aimed to search for this and other *TNFRSF1A* mutations in rheumatoid arthritis (RA), and to test for linkage.

Methods: The DNA of 100 trio families and 86 index cases of RA-affected sib-pair (ASP) families from the French Caucasian population were investigated by denatured high-performance liquid chromatography for *TNFRSF1A* mutations in exons 2 to 4. The test for association compared cases and controls (derived from un-transmitted parental chromosomes). The test for linkage relied on the transmission disequilibrium test (TDT) in trio families and cosegregation in ASP families.

Results: Only the R92Q mutation was detected – in 2 of the 100 index cases of trio families and in 5 (5.8%) of the index cases of ASP families, but also in 5% of the controls, showing no association with the disease. No RA linkage evidence was found in TDT and ASP RA families.

Conclusion: This *TNFRSF1A* investigation in RA from the French Caucasian population showed only the R92Q mutation, with a frequency of 4.5%, but no evidence for RA association or linkage to the disease. The R92Q mutation could be considered to be a low-penetrance variant.

INTRODUCTION

Rheumatoid arthritis (RA) is a complex disease for which a combination of risk alleles from different susceptibility genes predisposes to its development. Four genome scans in RA have proposed 12p13 as a susceptibility locus, which includes the tumour necrosis factor receptor (TNFR) p55 gene (*TNFRSF1A*).^{1–4} Mutations in exons 2 to 4 of the *TNFRSF1A* gene are dominantly inherited in *TNFRSF1A*-associated periodic syndrome (TRAPS, MIM#142680), demonstrating the involvement of *TNFRSF1A* in auto-inflammatory syndromes. Aksentijevich *et al.* have suggested that the *TNFRSF1A* R92Q mutation (rs4149584) that is associated with TRAPS could be involved in non-TRAPS arthritis, as the mutation was found in 5.2% of 135 patients with early arthritis.⁵ We have recently reported a negative association between the *TNFRSF1A* +36A/A genotype and RA, an association that is restricted to familial RA.⁶ The *TNFRSF1A* +36A/G single nucleotide polymorphism (rs767455) is characterized by an absence of amino-acid substitution, suggesting that the +36G allele is not the genetic factor *per se*, but that it is in linkage disequilibrium with it. Following these data, the aim of this study was to search for *TNFRSF1A* mutations in exons 2 to 4 and to test them for linkage to RA.

PATIENTS AND METHODS

A family-based association study was conducted to investigate the *TNFRSF1A* TRAPS-causing mutations in RA. All individuals provided informed consent, and the Ethics Committee of the Hôpital Bicêtre approved the study. Transmission Disequilibrium Test (TDT) RA families (one RA affected patient and both parents) were recruited through a national media campaign, followed by selection of individuals fulfilling the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 revised criteria for RA.⁷ Inclusion criteria for the 100 French Caucasian families evaluated here, that are investigated for various candidate genes, were the participation of one RA patient and both parents, as well as a European Caucasian origin of the family, with the four grandparents being from that population. Excluded were families with an additional sibling with RA or patients with RA who were younger than 18 years. Characteristics of both RA samples were previously reported.⁶

Genomic DNA used for genotyping was purified from fresh peripheral blood leukocytes by standard methods. Screening for *TNFRSF1A* mutations situated in exons 2, 3 and 4 was performed using a denatured high-performance liquid chromatography (dHPLC) method (WAVE DNA fragment analysis system; Transgenomic). The following oligonucleotides were used for PCR amplification: exon 2, 5'-AGGACTTGAGCC AGGGAAGT-3' (sense) and 5'-ACTTTGCTGTCTCTCTGGG-3' (antisense); exon 3, 5'-GGGCTCCTTCCTTGTTCT-3' (sense) and 5'-CTGACTCTCCTGCCTGTGC-3' (antisense); exon 4, 5'-TGCAGGACTCATACCCCATC-3' (sense) and 5'-CTTGGCCT CAGGAGAGCTG-3' (antisense). WaveMaker software was used to predict the mean melting temperature of each PCR fragment and the appropriate linear acetonitrile gradient necessary to distinguish heteroduplexes and homoduplexes.⁸ The dHPLC gradient conditions were 61°C for exon 2, 62.5°C for exon 3 and 61.9°C for exon 4, with acetonitrile gradients of 54–62%, 51–62% and 50–59% of buffer B, respectively. Samples showing abnormal elution profile were re-amplified with the same oligonucleotides from genomic DNA for direct sequencing.

Linkage and association analysis were performed using TDT⁹ and genotype relative risk (GRR) tests.¹⁰ *P* values less than 0.05 were considered to be significant.

RESULTS

Screening for *TNFRSF1A* mutations revealed only the R92Q mutation. Of the 100 TDT families, 2 RA index cases, 1 of the 6 RA-affected parents and 5 of the 200 control chromosomes

Abbreviations: dHPLC, denatured high-performance liquid chromatography; GRR, genotype relative risk; RA, rheumatoid arthritis; TDT, Transmission Disequilibrium Test; TNFR, tumour necrosis factor receptor; TRAPS, *TNFRSF1A*-associated periodic syndrome

Table 1 *TNFRSF1A**R92Q genotype distribution in ASP RA families

ASP RA families	Sex	Phenotype	<i>TNFRSF1A</i> *R92Q genotypes
Family #1			
Index	F	RA	R92Q
Sib 1	M	Healthy	R92Q
Sib 2	F	RA	R92R
Sib 3	M	Healthy	R92R
Sib 4	F	RA	R92R
Sib 5	F	Healthy	R92R
Sib 6	F	Healthy	R92Q
Family #2			
Index	F	RA + Sjögren	R92Q
Sib 1	M	RA	R92Q
Sib 2	F	Healthy	R92R
Sib 3	F	Healthy	R92Q
Family #3			
Index	M	RA	R92Q
Sib 1	M	Healthy	R92R
Sib 2	F	Healthy	R92R
Sib 3	M	Healthy	R92Q
Sib 4	F	RA	R92R
Family #4			
Index	M	RA + Sjögren	R92Q
Sib 1	F	RA	R92R
Sib 2	M	Healthy	R92Q
Family #5			
Index	M	RA	R92Q
Sib 1	F	Healthy	Not available
Sib 2	M	Healthy	Not available
Sib 3	M	Healthy	Not available
Sib 4	F	RA	Not available

F, female; M, male; RA, rheumatoid arthritis

(derived from un-transmitted parental chromosomes) presented the heterozygous mutation. The R92Q mutation was *de novo* for one of the two TDT RA index cases, as both parents were 92R/R. TDT analysis showed no excess of transmission of the *TNFRSF1A**92Q allele. GRR analysis found no excess of genotype carrying the R92Q substitution. Following the previously reported negative association between a *TNFRSF1A* genotype and RA, which was restricted to the multiplex RA sample, we also investigated the 86 RA index cases from the previously multiplex RA sample used.⁶ We observed an increase in the frequency of the *TNFRSF1A* R92Q mutation in familial RA, as 5 of the 86 ASP RA index cases carried the R92Q mutation (5.8%). However, this increase in frequency did not differ with the frequency of controls (5%). As observed in the TDT RA sample, no other TRAPS-causing mutations were detected in the ASP RA sample. Following these results, the sib-pairs of each ASP RA index case carrying the R92Q mutation were also investigated (DNA was not available the ASP family #5). No aggregation between the R92Q mutation and RA was observed (see table 1). No particular phenotype was observed in RA patients carrying the R92Q mutation (data not shown).

DISCUSSION

In the present study, we observed a lack of association between the *TNFRSF1A* TRAPS-causing mutation and RA. The *TNFRSF1A**92Q allele frequency found in RA patients was not different from that observed in controls (2.27% vs. 2.5%). Of the *TNFRSF1A* mutations reported to be associated with TRAPS, P46L and R92Q are likely to have the lowest penetrance for TRAPS, each having a frequency of ~1% in the control population, suggesting that both substitutions are low-penetrance mutations.⁵ The frequency of the *TNFRSF1A**92Q allele in our controls was higher than those previously reported in North American (0.95% in controls of Caucasian origin) and European (1.32% in controls of Caucasian origin) studies.^{5 11} However, in good agreement with our findings, D'Osualdo *et al.* have

recently reported a frequency of the *TNFRSF1A**92Q allele in the Italian population similar to that observed in our control population (2.25%).¹² Given the current hypothesis that complex genetic diseases probably arise from a combination of common low-penetrance mutations or polymorphisms, Hull *et al.* have suggested that the *TNFRSF1A* R92Q mutation could be a genetic contributory factor to a cluster of inflammatory disorders.¹³ To strengthen the possible contribution of *TNFRSF1A* R92Q mutation in non-TRAPS phenotypes, an association was recently reported with a particular phenotype of Behcet's disease.¹¹ Following the hypothesis of an involvement of *TNFRSF1A* R92Q as a genetic factor for various autoimmune phenotypes, we investigated the familial aggregation of autoimmune disorders in families for whom one parent carried the *TNFRSF1A* R92Q substitution. No aggregation between the R92Q substitution and autoimmune phenotype was observed (data not shown).

Aksentijevich *et al.* found no defect in *TNFRSF1A* R92Q shedding in vitro; however, in vivo, they observed that soluble *TNFRSF1A* levels did not increase with attacks.⁵ More recently, D'Osualdo *et al.* have confirmed a defect in the shedding in TRAPS patients with cysteine mutations, except for the R92Q substitution.¹² The same authors also observed that patients with TRAPS with a worst disease course carried *TNFRSF1A* cysteine mutations, which are associated with a defect in TNF-induced neutrophil apoptosis. Conversely, patients with TRAPS carrying the R92Q substitution have a milder disease course and no defect in TNF-induced neutrophil apoptosis.¹³ Similar results were observed in a recent French study of patients with TRAPS.¹⁴

In conclusion, TRAPS-causing mutations are not associated with RA in this French Caucasian population. The *TNFRSF1A* R92Q mutation could be considered to be a low-penetrance variant, which could have a weak contribution to auto-inflammatory diseases. The influence of the R92Q variation in RA should be investigated before concluding that it does not contribute to RA genetics.

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