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THE *TNFRSF1A R92Q* MUTATION IS FREQUENT IN RHEUMATOID ARTHRITIS BUT SHOW NO EVIDENCE FOR ASSOCIATION NOR LINKAGE WITH THE DISEASE.

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ABSTRACT

Objective : *TNFRSF1A* mutations cause TRAPS (MIM#142680). A recent study suggested that the R92Q mutation was associated with polyarthritis. We aimed at searching for this and other *TNFRSF1A* mutations in RA, to be tested for linkage.

Material and methods : the 386 DNA of 100 trio families and 86 index cases of RA affected sib-pair (ASP) families from the French Caucasian population were investigated by dHPLC (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 5 (5.8%) of the index cases of ASP families, but also 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 nor linkage to the disease. The R92Q mutation could be considered as 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 the development. Four genome scans in RA have pointed 12p13 as a susceptibility locus, which includes the tumor necrosis factor receptor (TNFR) p55 gene (*TNFRSF1A*) [1-4]. Mutations in exon 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) 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, association restricted to familial RA [6]. The *TNFRSF1A* +36A/G single nucleotide polymorphism is (rs767455) characterized by an absence of amino acid substitution, suggesting that the +36G allele is not the genetic factor *per se*, but in linkage disequilibrium with it. Following those data, the aim of this study was search for exon 2 to 4 *TNFRSF1A* mutations and to test those 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 1 RA patient and both parents, as well as a European Caucasian origin of the family, with the 4 grandparents being from that population. Excluded were families with an additional sibling with RA or RA patients 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 dHPLC method (WAVE DNA fragment analysis system, Transgenomic). Following oligonucleotides were used for PCR amplification: exon 2, 5'-AGGACTTGAGCCAGGGAAGT-3' (sense) and 5'-ACTTTGCTGTCTCTCCTGGG-3' (antisense); exon 3, 5'-GGGCTCCTTCCTTGTGTTCT-3' (sense) and 5'-CTGACTCTCCTGCCTGTGC-3' (antisense); exon 4, 5'-TGCAGGACTCATACCCCATC-3'(sense) and 5'-CTTGGCCTCAGGAGAGCTG-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 reamplified with same oligonucleotides from genomic DNA for direct sequencing.

Linkage and association analysis were performed using TDT [9] and genotype relative risk (GRR) tests [10]. *P* values less than 0.05 were considered significant.

RESULTS

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

chromosomes (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, 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 of 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 of 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 those results, the sibpairs of each ASP RA index cases carrying the R92Q mutation were also investigated (DNA was not available the ASP family N°5). No aggregation between the R92Q mutation and RA was observed (see Table). 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 than that observed in controls (2.27% versus 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 studies (1.32% in controls of Caucasian origin) [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%). Given the current hypothesis that complex genetic diseases probably arise from a combination of common low penetrance mutation or polymorphisms, Hull *et al* have suggested that *TNFRSF1A* R92Q mutation could be a genetic contributively factor to a cluster of inflammatory disorders [12]. 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 which 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. The same authors also observed that TRAPS patients with a worst disease course carried *TNFRSF1A* cysteine mutations, which are associated with a defect in TNF-induced neutrophil apoptosis. Oppositely, TRAPS patients 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 TRAPS patients [14].

In conclusion, TRAPS-causing mutations are not associated with RA in this French Caucasian population. The *TNFRSF1A* R92Q mutation could be considered as a low-penetrance variant, which could have a weak contribution to auto-inflammatory diseases. The influence of the

R92Q variation in RA course should be investigated before concluding to the absence of its contribution in RA genetics.

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TNFRSF1A*R92Q GENOTYPES DISTRIBUTION IN ASP RA FAMILIES

ASP RA families	Sex	Phenotype	TNFRSF1A*R92Q genotypes
Family #1			
Index	F	RA	R92Q
Sib1	M	Healthy	R92Q
Sib2	F	RA	<i>R92R</i>
Sib3	M	Healthy	<i>R92R</i>
Sib4	F	RA	<i>R92R</i>
Sib5	F	Healthy	<i>R92R</i>
Sib6	F	Healthy	R92Q
Family #2			
Index	F	RA + Sjögren	R92Q
Sib1	M	RA	R92Q
Sib2	F	Healthy	<i>R92R</i>
Sib3	F	Healthy	R92Q
Family #3			
Index	M	RA	R92Q
Sib1	M	Healthy	<i>R92R</i>
Sib2	F	Healthy	<i>R92R</i>
Sib3	M	Healthy	R92Q
Sib4	F	RA	<i>R92R</i>
Family #4			
Index	M	RA + Sjögren	R92Q
Sib1	F	RA	<i>R92R</i>
Sib2	M	Healthy	R92Q
Family #5			
Index	M	RA	R92Q
Sib1	F	Healthy	<i>Not available</i>
Sib2	M	Healthy	<i>Not available</i>
Sib3	M	Healthy	<i>Not available</i>
Sib4	F	RA	<i>Not available</i>

RA: rheumatoid arthritis

M: male

F: female