

# The *MMP2* rs243865-T allele is not a major genetic factor for rheumatoid arthritis in the French Caucasian population

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## Summary

The *MMP2* rs243865-T allele was recently suggested to be associated with rheumatoid arthritis (RA) in a case-control study. *MMP2* is a positional RA candidate gene. Our aim was to test rs243865 in a French family based study. No significant result was shown. The *MMP2* rs243865-T allele is not a major rheumatoid arthritis genetic factor in this population.

## Introduction

Rheumatoid arthritis (RA) is a common human systemic autoimmune disease, for which previous studies have suggested the importance of genetic factors (Seldin *et al.*, 1999). Two genes have been established so far and confirmed with family-based studies, *HLA-DRB1* and *PTPN22* (Deighto *et al.*, 1989; Dieudé *et al.*, 2005). A genome scan performed in a French Caucasian population suggested 19 non-*HLA* regions (Osorio *et al.*, 2004). The *MMP2* gene is located in one of these regions (2q33), and was recently suggested as a susceptibility gene (rs243865-T allele), in a case-control Spanish study (Rodriguez-Lopez *et al.*, 2006). This gene is a good functional RA candidate because of its role in the degradation of extracellular matrix of the cartilage. Furthermore, the rs243865 C/T single nucleotide polymorphism (SNP) is located in the promoter sequence, and the T allele displays a lower promoter activity (Goldbach-Mansky *et al.*, 2000; Price *et al.*, 2001; Buisson-Legendre *et al.*, 2004). The case-control approach cannot avoid imperfect matching between cases and controls, and the association may consequently be 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.

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The aim of our study was to test this SNP in the hypothesis of a major, clinically relevant genetic factor supporting the RA susceptibility locus on 2q33, using a family-based analysis from the French Caucasian population.

## Materials and methods

DNA samples were from 100 French Caucasian Trio families (one patient and both parents) with the four grandparents of French Caucasian origin (the characteristics of the sample population are presented in Table 1). RA families were recruited through a French media campaign followed by selection of individuals who fulfilled the 1987 American College of Rheumatology criteria (Arnett *et al.*, 1988). All subjects provided informed consent, and the ethics committee of Hôpital Bicêtre (Kremlin-Bicêtre, France), approved the study. DNA was isolated and purified from whole blood according to standard protocols (Sambrook *et al.*, 1989).

Genotyping was performed using a Taqman 5' allelic discrimination assay (assay C\_3225943-10, Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocol. Genotypes of 30 individuals were checked again randomly for quality control.

Association and linkage were examined using three methods. Affected family-based controls (AFBAC) were used to compare transmitted and untransmitted allelic frequencies across all families. The transmission disequilibrium test (TDT) was used to detect linkage through preferential transmission of one allele to the affected subjects. The genotype relative risk (GRR) was calculated to compare the genotypic distribution in patients and

**Table 1.** Characteristics of rheumatoid arthritis index cases (*n*: number of cases)

	Sample ( <i>n</i> = 100)
Females	87
Mean age at disease onset (years)	32 ± 10
Mean disease duration (years)	18 ± 7
Patients with erosions (ER+)	90
Patients seropositive	81
Patients carrying at least	78

One *HLA-DRB1* shared epitope allele.

**Table 2.** Affected family-based controls (AFBAC) and transmission disequilibrium test (TDT) analysis for rs243865 (*n*: number of heterozygote parents)

Allele	AFBAC			TDT		
	RA index cases	Controls	<i>P</i>	% of Trans	<i>n</i>	<i>P</i>
<i>rs243865-T</i>	0.242	0.212	0.4	54.8	62	0.4
ER + cases	0.253	0.242	0.8	52.5	59	0.5

RA, rheumatoid arthritis.

**Table 3.** Genotype relative risk (GRR) analysis for rs243865 (ER + cases)

Genotypes	RA index cases	Controls	<i>P</i>
<i>C/C</i>	58 (51)	60 (49)	0.7 (0.6)
<i>C/T</i>	33 (30)	34 (35)	
<i>T/T</i>	7 (7)	4 (4)	

RA, rheumatoid arthritis.

controls (Lathrop, 1983; Spielman *et al.*, 1993; Thomson, 1995). The significance of *P*-value was assessed at 5% and led to a replication test in another 100 families sample if at least one test had a significant result. A study of the erosive subgroup (ER+) of patients with RA was also done, according to the *MMP2* gene function.

Power calculation: with an allele frequency of the *rs243865-T* of 24.5% and 20.3%, in RA index cases and controls, respectively (Spanish study allelic frequencies), a sample size of 100 patients and 100 controls, and the arc sinus transformation method precedently described by Garnier *et al.* (2006), we had a 63.7% power to detect a significant association ( $P < 0.05$ ).

## Results and discussion

The observed rs243865 genotype frequencies were in accordance with the Hardy–Weinberg equilibrium in ‘virtual controls’, constituted by parental untransmitted alleles to RA index cases and controls. We observed neither significant linkage nor association between rs243865 and RA. We found, however, a trend for the *T* allele (TDT 54.8% of transmission vs. 50%,  $P = 0.4$ , AFBAC RA index cases 24.2% vs. controls 21.2%,  $P = 0.4$ ) and for the *T/T* genotype (seven RA index cases vs. four controls,  $P = 0.7$ ). We did not find any more evidence in the ER + subgroup (Tables 2 and 3).

The aim of this study was to detect a major RA susceptibility allele located at the 2q33 susceptibility locus and suggested in a case–control study. Our results allow to exclude the *rs243865-T* allele as a major genetic factor in the French Caucasian population, but a minor significant RA association cannot be totally excluded. However, a 80% detection power (based on the allelic frequencies of

our study) would require 1200 Trio families ( $P < 0.05$ ) and several thousand Trio families for a definitive association ( $P < 10^{-6}$ ).

In conclusion, this study provides evidence that the *MMP2 rs243865-T* allele is not involved in the RA susceptibility locus on 2q33 and is not a major, clinically relevant RA susceptibility genetic factor in the French Caucasian population.

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