Lack of association of the *GSDMB* gene at locus 17q12 with predisposition to rheumatoid arthritis

Sirs,

Rheumatoid arthritis (RA) is a chronic inflammatory disease, in which pathogenesis many genetic, environmental and hormonal factors have been implicated. To date, the known genetic factors account for 60% of the genetic basis of the disease (1) implicated 37 candidate molecular pathways (2) and therefore more genetic association studies are needed to explore the additional polymorphisms in RA predisposition with probably lower penetrance. In a recent multiethnic approach for the identification of RA susceptibility loci, two chromosome loci, the 1p36 and the 17q12, were revealed as most likely associated with RA manifestation (3). Subsequently, bioinformatic analyses identified *TNFRSF14-MMEL1* at the 1p36 locus and *IKZF3-ORMDL3-GSDMB* at the 17q12 locus as the most probably related genes with RA liability (3). Since the genetic association of locus TNFRSF14-MMEL1 with RA has been reported previously (4), our interest has focused on genes *IKZF3-ORMDL3-GSDMB* at locus 17q12.

At locus 17q12 the most associated polymorphism with RA, in this multi-ethnic approach, was the rs2872507. This intergenic variant was found to be in linkage disequilibrium (LD) with the missense polymorphism rs2305479 which alters a glycine to arginine at codon 299 in the *GSDMB* (gasdermin B) gene and predicted to be damaging (3). Therefore, in the present study, we investigated, for the first time, the plausible probably association of the *GSDMB* gene with RA predisposition by genotyping polymorphism rs2305479.

One hundred and eighty-five unrelated RA patients, who satisfied the American College of Rheumatology criteria, and 147 ethnic-matching healthy volunteers were enrolled in the study. Genomic DNA was extracted from peripheral blood lymphocytes according to the standard salt extraction procedure. GSDMB polymorphism rs2305479 was amplified using the following primer pair: rs2305479F: 5'- CTA AGA AAG CCT TGG GGC AGA -3', rs2305479R: 5'- TCC AGA ATG GCT TTT GCA CG -3'. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was conducted using the restriction endonuclease *Msp I*. The SPSS statistical package was used to test differences in polymorphism distribution between RA patients and controls (Pearson's chi-square). Furthermore, the odds ratio (OR) with a confidence interval (CI) of 95% was calculated. A difference at $p<0.05$ was considered as statistically significant.

Table I. Genotypes' and alleles' distribution of GSDMB gene polymorphism rs2305479 in rheumatoid arthritis (RA) patients and controls.

	GSDMB rs2305479		
Genotypes	GG	GA	AA
RA n=185 (%)	41 (22.2)	100 (54.1)	44 (23.8)
Controls n=147 (%)	31 (21.1)	82 (55.8)	34 (23.1)
p-value		0.889	
Alleles	G		A
RA n=370 (%)	182 (49.2)		188 (50.8)
Controls n=294 (%)	144 (49)		150 (51)
p-value		0.957	
OR (95% CI)		0.992 (0.730–1.374)	

The studied polymorphism was found in Hardy-Weinberg equilibrium in both RA patients (p=0.268) and controls (p=0.159). No statistical significant difference was observed in genotypes' and alleles' rs2305479 distribution between RA patients and controls as shown in Table I.

GSDMB encodes a member of the gasdermin-domain-containing protein family and is highly expressed in the thymus, lymph nodes, and $CD4^+$ and $CD8^+$ T cells, and consequently it could be related to RA susceptibility. Previously, the rs2872507 polymorphism located at site 17q12 has been associated with RA (3, 5). Trying to find out the causative gene of this chromosome region, we genotyped the missense GSDMB polymorphism rs2305479 being in strong LD with rs2872507. However, no association with RA predisposition was revealed in our group of patients.

In this chromosome locus another gene has been mapped, the *ORMDL3* (orosomucoid-like 3) of which polymorphisms have been associated with asthma, a chronic airway inflammation (6, 7). *ORMDL3* gene is highly associated with *GSDMB*, not only because both are mapped at locus 17q12-21, but also because polymorphisms in the *GSDML* (gasdermin-like) gene and the transcript levels of ORMDL3 have been significantly associated (7-9). Therefore, taking into account the results of the present study and the fact that polymorphism rs2872507 is in LD with polymorphisms in *GSDMB* and genetic variants of *GSDMB* with others of *ORMDL3* (3, 6, 7), we should search for *ORMDL3* polymorphism association with RA predisposition in future studies so as to resolve the question what is the causative RA gene at chromosome locus 17q12-21.

A. CHATZIKYRIAKIDOU[1,2], *Lecturer in Med. Genetics*
P.V. VOULGARI[2], *MD, Assoc. Prof.*
A.A. DROSOS[2], *MD, FACR, Prof.*

[1]Laboratory of General Biology and Genetics, Medical School, Aristotle University of Thessaloniki, Greece, and [2]Rheumatology Clinic, Department of Internal Medicine, Medical School, University of Ioannina, Greece.

Please address correspondence to:
Prof. Alexandros A. Drosos, Rheumatology Clinic, Department of Internal Medicine, Medical School, University of Ioannina, 45110 Ioannina, Greece.
E-mail: adrosos@cc.uoi.gr

Competing interests: none declared.

References

1. CHATZIKYRIAKIDOU A, VOULGARI PV, LAMBROPOULOS A, DROSOS AA: Genetics in rheumatoid arthritis beyond HLA genes: What meta-analyses have shown? *Semin Arthritis Rheum* 2013; 43: 29-38.
2. SONG GG, BAE SC, LEE YH: Pathway analysis of genome-wide association studies on rheumatoid arthritis. *Clin Exp Rheumatol* 2013; 31: 566-74.
3. KURREEMAN FA, STAHL EA, OKADA Y *et al*.: Use of a multiethnic approach to identify rheumatoid- arthritis-susceptibility loci, 1p36 and 17q12. *Am J Hum Genet* 2012; 90: 524-32.
4. RAYCHAUDHURI S, REMMERS EF, LEE AT *et al*.: Common variants at CD40 and other loci confer risk of rheumatoid arthritis. *Nat Genet* 2008; 40: 1216-23.
5. STAHL EA, RAYCHAUDHURI S, REMMERS EF *et al*.: Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. *Nat Genet* 2010; 42: 508-14.
6. MADORE AM, TREMBLAY K, HUDSON TJ, LAPRISE C: Replication of an association between 17q21 SNPs and asthma in a French-Canadian familial collection. *Hum Genet* 2008; 123: 93–5.
7. MOFFATT MF, KABESCH M, LIANG L *et al*.: Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature* 2007; 448: 470–3.
8. HALAPI E, GUDBJARTSSON DF, JONSDOTTIR GM *et al*.: A sequence variant on 17q21 is associated with age at onset and severity of asthma. *Eur J Hum Genet* 2010; 18: 902-8.
9. LLUIS A, SCHEDEL M, LIU J *et al*.: Asthma-associated polymorphisms in 17q21 influence cord blood ORMDL3 and GSDMA gene expression and IL-17 secretion. *J Allergy Clin Immunol* 2011; 127: 1587-94.