Reply

Sirs,

We greatly appreciate the interest shown by Brenol *et al*. for our data showing for first time that some interactions between NOS gene polymorphisms and HLA-DRB1 alleles confer and increased risk of developing cardiovascular events in patients with rheumatoid arthritis (RA) (1).

As pointed out by Dr Brenol *et al*., the different genetic backgrounds of the populations may explain differences in terms of NOS gene polymorphism associations with susceptibility to RA.

With respect to the increased risk of accelerated atherogenesis observed in patients with RA, we previously described an association between endothelial dysfunction, which is an early step in the atherogenesis process (2), and HLA-DRB1*04 shared epitope alleles, in particular with HLA-DRB1*0404 (3). More recently, we also confirmed an increased risk of developing cardiovascular events and cardiovascular mortality in patients with RA carrying these HLA-DRB1*04 shared epitope alleles, especially HLA-DRB1*0404 (4).

RA is a polygenic disease and, besides the well-known association with genes lying within the MHC region, there is growing body of evidence supporting the contribution of additional genes located outside this region (5). In this regard, gene-gene interaction is also a plausible explanation for an increased risk of susceptibility and severity associated to this chronic inflammatory autoimmune disease.

In our former study we assessed interactions between NOS2A promoter CCTTT repeat microsatellite or NOS3 gene polymorphisms and HLA-DRB1 for the risk of developing cardiovascular events in patients with RA (1). We reported an increased frequency of cardiovascular events in patients with RA who carried the HLA-DRB1*0404 allele and were homozygous for the NOS3 (-786) TT genotype (OR: 9.06 [95% CI: 1.29–63.37]; p=0.03). It was also the case for RA patients who were homozygous for the presence of long NOS2A alleles and carried the HLA-DRB1*0404 allele (OR: 11.7 [95% CI: 1.53–88.4]; p=0.02) (1).

In assessing the interaction between HLA-DRB1*04-shared epitope positive alleles, in particular HLA-DRB1*0404, and the NOS3 (-786) TT genotype, we should consider two very different hypotheses:

(a) NOS3 (-786) TT genotype increases the risk of cardiovascular events in RA patients carrying the HLA-DRB1*0404 allele.

(b) HLA-DRB1*0404 allele increases the risk for cardiovascular events in RA patients with NOS3 (-786) TT genotype.

The results shown in Table III of our former manuscript (1) do strongly support hypothesis (b), because high odds ratios were found when RA patients different from those carrying HLA-DRB1*04-shared epitope alleles were used as reference.

The comment raised by Dr Brenol *et al*. seems to refer to hypothesis (a), as they include evidence on the lack of functional alteration in patients with TT genotype. Therefore, their comment does not contradict our results.

In conclusion, our data support a role of HLA-DRB1*0404 allele to increase the risk of cardiovascular events in RA patients carrying NOS3 (-786) TT genotype. More importantly, our data emphasise, for the first time, the additive effect of two genes located in different regions in the increased risk of accelerated atherogenesis and cardiovascular events described in patients with RA.

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