

## Questions on 'Sequencing of the MHC region defines HLA-DQA1 as the major genetic risk for seropositive rheumatoid arthritis in Han Chinese population' by Guo *et al*

We read with great interest the paper by Guo *et al*<sup>1</sup> addressing the HLA association with seropositive rheumatoid arthritis (RA) in Han Chinese. The authors reported that aspartic acid at position 160 of HLA-DQ $\alpha$ 1 (HLA-DQ $\alpha$ 1:160D) was the major risk factor. It was accompanied by asparagine at position 37 of HLA-DR $\beta$ 1 (HLA-DR $\beta$ 1:37N), which was protective. These results were obtained by targeted sequencing in 961 cases and 1812 controls distributed in discovery and validation stages. The underlying assumption is that sequencing had uncovered new susceptibility HLA alleles. Specifically, HLA-DQ $\alpha$ 1 has not previously been associated with RA, whereas the most associated HLA alleles and amino acids were those included in the shared epitope (SE) of HLA-DRB1.<sup>2-4</sup> SE alleles that have been associated with increased RA risk in all the ethnic groups analysed including the Han Chinese and other Asian ethnicities.<sup>2-5</sup> The new results are, therefore, of an extraordinary novelty and need to be considered with attention.

A careful analysis shows reasons for concern due to internal inconsistencies in the Guo *et al* study.<sup>1</sup> These inconsistencies include amino acids at DQ $\alpha$ 1:160 that do not sum up: the frequencies of the three amino acids (D, A and S) were 0.20, 0.22 and  $\approx$ 0.02 in controls and 0.36, 0.37 and  $\approx$ 0.01 in patients with RA, respectively (table 1). The three amino acids did not add up to 1.0 as required given that they are the only amino acids at this position. Also, the OR in cases/controls of the DQ $\alpha$ 1:160 amino acids was inconsistently described: two of the amino acids were described as increased in patients with RA, DQ $\alpha$ 1:160D with OR=2.36 and DQ $\alpha$ 1:160A with OR=2.27 (table 1). These ORs are impossible considering the low frequency of the third (DQ $\alpha$ 1:160S) amino acid. None of these inconsistencies can be attributed to a typographical error because they appear in multiple places. In addition, the DR $\beta$ 1:37N amino acid was reported as associated with protection from RA with OR=0.49 and  $p=5.81 \times 10^{-16}$ , but its frequency was identical in patients with RA and controls (table 1). This puzzling result is unlikely to be a typographical error because the equality between patients and controls is reported in three supplementary tables and because the omnibus test performed by Guo *et al* did not find DR $\beta$ 1:37N among the DRB1 amino acids associated with RA (supplementary table 10 in Guo *et al*). On the contrary, the most associated DRB1 amino acids (page 776 and supplementary

table 10 in Guo *et al*) were the same reported in other studies that correspond to the SE, which are the 11 and 13 amino-acid positions and the DRB1\*04:05 allele.<sup>2-5</sup> Besides these internal inconsistencies, the frequency of the DQ $\alpha$ 1 alleles containing the DQ $\alpha$ :160A amino acid was much lower in Guo *et al* than in other studies including those done in Asians (table 1).<sup>6</sup> These inconsistencies are worrisome and ask for clarification.

**Cristina Regueiro, Antonio Gonzalez** 

Experimental and Observational Rheumatology, Instituto Investigacion Sanitaria-Hospital Clinico Universitario de Santiago, Santiago de Compostela, Spain

**Correspondence to** Dr Antonio Gonzalez, Experimental and Observational Rheumatology, Instituto Investigacion Sanitaria-Hospital Clinico Universitario de Santiago, Santiago de Compostela 15706, Spain; agmartinezp@ser.es

**Contributors** CR and AG designed and wrote this letter.

**Funding** This study was funded by Instituto de Salud Carlos III (P117/O1606).

**Competing interests** None declared.

**Patient consent for publication** Not required.

**Provenance and peer review** Not commissioned; internally peer reviewed.

© Author(s) (or their employer(s)) 2020. No commercial re-use. See rights and permissions. Published by BMJ.



**To cite** Regueiro C, Gonzalez A. *Ann Rheum Dis* Epub ahead of print: [please include Day Month Year]. doi:10.1136/annrheumdis-2020-217031

Received 21 January 2020

Accepted 24 January 2020

*Ann Rheum Dis* 2020;0:1. doi:10.1136/annrheumdis-2020-217031

**ORCID iD**

Antonio Gonzalez <http://orcid.org/0000-0002-2624-0606>

### REFERENCES

- Guo J, Zhang T, Cao H, *et al*. Sequencing of the MHC region defines HLA-DQA1 as the major genetic risk for seropositive rheumatoid arthritis in Han Chinese population. *Ann Rheum Dis* 2019;78:773-80.
- Raychaudhuri S, Sandor C, Stahl EA, *et al*. Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. *Nat Genet* 2012;44:291-6.
- Liu X, Guo J, Jia Y, *et al*. HLA-DRB1 shared epitope-dependent DR-DQ haplotypes are associated with both anti-CCP-positive and -negative rheumatoid arthritis in Chinese Han. *PLoS One* 2013;8:e71373.
- Okada Y, Kim K, Han B, *et al*. Risk for ACPA-positive rheumatoid arthritis is driven by shared HLA amino acid polymorphisms in Asian and European populations. *Hum Mol Genet* 2014;23:6916-26.
- Reynolds RJ, Ahmed AF, Danila MI, *et al*. HLA-DRB1-associated rheumatoid arthritis risk at multiple levels in African Americans: hierarchical classification systems, amino acid positions, and residues. *Arthritis Rheumatol* 2014;66:3274-82.
- González-Galarza FF, Takeshita LYC, Santos EJM, *et al*. Allele frequency net 2015 update: new features for HLA epitopes, KIR and disease and HLA adverse drug reaction associations. *Nucleic Acids Res* 2015;43:D784-8.

**Table 1** Inconsistencies in the frequencies of the top associated amino acids from Guo *et al*<sup>1</sup>

Amino acids	Guo <i>et al</i>			Other studies*		
	Frequency†			Hong Kong Chinese	Koreans‡	Japanese
	Controls	Patients with RA	OR‡	n=1064	n=1209	n=3078
DQ $\alpha$ 1:160D	0.20	0.36	2.36	0.21	0.22	0.31
DQ $\alpha$ 1:160A	0.22	0.37	2.27	0.78	0.76	0.66
DQ $\alpha$ 1:160S	0.02	0.01		0.01	0.02	0.03
DR $\beta$ 1:37N	0.23	0.23	0.49			

\*Taken from the Allele Frequencies Net Database (<http://allelefrequencies.net>).

†Frequencies taken from Guo *et al*. Figure 3 and supplementary tables 3, 5 and 8 for DQ $\alpha$ 1 amino acids and from supplementary tables 4, 6 and 8 for DR $\beta$ 1:37N.

‡OR reported in pages 775 and 776 of Guo *et al* for DQ $\alpha$ 1 and DR $\beta$ 1:37N, respectively.

§Results for Koreans were combined from four studies.

RA, rheumatoid arthritis.