Questions on 'Sequencing of the MHC region defines *HLA-DQA*1 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*[1] addressing the HLA association with seropositive rheumatoid arthritis (RA) in Han Chinese. The authors reported that aspartic acid at position 160 of HLA-DQα1 (HLA-DQα1:160D) was the major risk factor. It was accompanied by asparagine at position 37 of HLA-DRβ1 (HLA-DRβ1:37N), 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-DQα1 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.[2–4] SE alleles that have been associated with increased RA risk in all the ethnic groups analysed including the Han Chinese and other Asian ethnicities.[2–5] 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.[1] These inconsistencies include amino acids at DQα1:160 that do not sum up: the frequencies of the three amino acids (D, A and S) were 0.20, 0.22 and ≈0.02 in controls and 0.36, 0.37 and ≈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 DQα1:160 amino acids was inconsistently described: two of the amino acids were described as increased in patients with RA, DQα1:160D with OR=2.36 and DQα1:160A with OR=2.27 (table 1). These ORs are impossible considering the low frequency of the third (DQα1:160S) amino acid. None of these inconsistencies can be attributed to a typographical error because they appear in multiple places. In addition, the DRβ1:37N amino acid was reported as associated with protection from RA with OR=0.49 and $p=5.81\times10^{-16}$, 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 DRβ1:37N 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.[2–5] Besides these internal inconsistencies, the frequency of the DQA1 alleles containing the DQα:160A amino acid was much lower in Guo *et al* than in other studies including those done in Asians (table 1).[6] These inconsistencies are worrisome and ask for clarification.

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Table 1 Inconsistencies in the frequencies of the top associated amino acids from Guo *et al*[1]

	Guo *et al*			Other studies*		
	Frequency†			Hong Kong Chinese	Koreans§	Japanese
Amino acids	Controls	Patients with RA	OR‡	n=1064	n=1209	n=3078
DQα1:160D	0.20	0.36	2.36	0.21	0.22	0.31
DQα1:160A	0.22	0.37	2.27	0.78	0.76	0.66
DQα1:160S	0.02	0.01		0.01	0.02	0.03
DRβ1:37N	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 DQα1 amino acids and from supplementary tables 4, 6 and 8 for DRβ1:37N.
‡OR reported in pages 775 and 776 of Guo *et al* for DQα1 and DRβ1:37N, respectively.
§Results for Koreans were combined from four studies.
RA, rheumatoid arthritis.