Response to: '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*' by Regueiro and Gonzalez

We appreciate Dr Gonzalez's interest and comments on our recent publication 'Sequencing of the major histocompatibility complex (MHC) region defines human leukocyte antigen (HLA)-DQA1 as the major genetic risk for seropositive rheumatoid arthritis in Han Chinese population'.^{1,2} Dr Gonzalez's comments provide us with an opportunity to clarify and discuss the frequencies of amino-acids at position DQ α 1:160 and the protective association of DR β 1:37N in rheumatoid arthritis (RA), and to improve our study.

One of the concerns Dr Gonzalez expressed is the frequencies of three amino-acids (Asp (D), Ala (A) and Ser (S)) at DQ α 1:160 do not sum up to 1.0, that is, 0.20, 0.22 and $\approx$ 0.02 in controls; 0.36, 0.37 and $\approx$ 0.01 in RA patients. The explanation is that the 'minor frequencies' was set as default for all variants in PLINK. The original frequencies of three amino-acids (D, A and S) were 0.20, 0.78 (1–0.22) and $\approx$ 0.02 (the sum is 1.00) in controls and 0.36, 0.63 (1–0.37) and $\approx$ 0.01 (the sum is 1.00) in cases, respectively. In our original paper the frequencies of DQ α 1:160A in healthy controls were similar to those reported in other Asian studies.³ These results do not affect the calculation of *p* value, but do affect the odd ratio (OR) calculation. Indeed, by the omnibus test DQ α 1:160A showed a protective effect (OR=0.46, *p*= 2.72×10^{-35} , online supplementary table 10 in Guo *et al*).² We appreciate Dr Gonzalez *et al* for this important point and have made a correction for our publication, in which all variants have been presented according to original frequencies instead of minor frequencies.⁴

Regarding the protective effect of DR β 1:37N, although the identified amino-acid DR β 1:37N did not show any significant association in univariate regression analysis, it reached second strong statistical significance after conditioning on DQ α 1:160D in both discovery and validation stages, indicating an independent association. This phenomenon could be potentially explained by the Simpson's paradox, a striking observation that an association between two variables at the population-level might increase or decrease in quantity, or even change direction within the subgroups, depending on the set of variables being controlled,^{5,6} and has been reported in several genetic association studies.^{7,8} Notably, the DR β 1:11D also showed an independent protective effect and was in high linkage disequilibrium (LD) with DR β 1:37N (*r*²=0.62; online supplementary tables 8 and 9 in Guo *et al*).

Regarding other DRB1 variants, as the author indicated, by omnibus test we replicated the findings reported in previous studies,^{9–12} including the position 11 and 13 at DR β 1, and the allele DRB1*04:05. However, our study focused on single nucleotide polymorphisms (SNPs), classical HLA alleles and the individual amino-acid variants rather than amino-acid positions, because a particular amino-acid(s) may have potential biological function(s). Furthermore, different amino-acids at same position may insert different functions.¹³ Taking this into consideration, DQ α 1:160D remained the top association in omnibus test (OR=2.30, *p*= 1.82×10^{-38}) (online supplementary table 10 in Guo *et al*). Furthermore, consistent with our findings, Hirata *et al*¹⁴ have also reported that one of DQ α 1:160D encoding allele DQ α 1*0303 was a strong risk for susceptibility to RA in Japanese population (OR=2.65, *p*= 2.0×10^{-173} , shown in table 1 in Hirata *et al*).

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REFERENCES

- Regueiro C, Gonzalez A. 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*. *Ann Rheum Dis* 2020;annrheumdis-2020-217031.
- 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.
- 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.
- Correction: Sequencing of the MHC region defines *HLA-DQA1* as the major genetic risk for seropositive rheumatoid arthritis in Han Chinese population. *Ann Rheum Dis* 2020. doi:10.1136/annrheumdis-2018-214725corr1. [Epub ahead of print: 20 Apr 2020].
- Simpson EH. The interpretation of interaction in contingency tables. *J R Stat Soc Series B* 1951;13:238–41.
- Wagner CH. Simpson's paradox in real life. *The American Statistician* 1982;36:46–8.
- Patsopoulos NA, Barcellos LF, Hintzen RQ, *et al*. Fine-mapping the genetic association of the major histocompatibility complex in multiple sclerosis: HLA and non-HLA effects. *PLoS Genet* 2013;9:e1003926.
- Zhou F, Cao H, Zuo X, *et al*. Deep sequencing of the MHC region in the Chinese population contributes to studies of complex disease. *Nat Genet* 2016;48:740–6.

- 9 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.
- 10 Okada Y, Suzuki A, Ikari K, *et al.* Contribution of a non-classical HLA gene, HLA-DOA, to the risk of rheumatoid arthritis. *Am J Hum Genet* 2016;99:366–74.
- 11 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.
- 12 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.
- 13 Scally SW, Law S-C, Ting YT, *et al.* Molecular basis for increased susceptibility of Indigenous North Americans to seropositive rheumatoid arthritis. *Ann Rheum Dis* 2017;76:1915–23.
- 14 Hirata J, Hosomichi K, Sakaue S, *et al.* Genetic and phenotypic landscape of the major histocompatibility complex region in the Japanese population. *Nat Genet* 2019;51:470–80.