Response to: '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*' by Regueiro and Gonzalez

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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 DQα1:160 and the protective association of DRβ1:37N 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 DQα1:160 do not sum up to 1.0, that is, 0.20, 0.22 and ≈0.02 in controls; 0.36, 0.37 and ≈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 ≈0.02 (the sum is 1.00) in controls and 0.36, *0.63* (1–0.37) and ≈0.01 (the sum is 1.00) in cases, respectively. In our original paper the frequencies of DQα1:160A inhealthy 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 DQα1:160A showed a protective effect (OR=0.46, p=2.72x 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 DRβ1:37N, although the identified amino-acid DRβ1:37N did not show any significant association in univariate regression analysis, it reached second strong statistical significance after conditioning on DQα1:160D 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 DRβ1:11D also showed an independent protective effect and was in high linkage disequilibrium (LD) with DRβ1:37N (r2=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 DRβ1, 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, DQα1:160D remained the top association in omnibus test (OR=2.30, p=1.82x 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 DQα1:160D encoding allele *DQA1*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

1 Regueiro C, Gonzalez A. 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*. *Ann Rheum Dis* 2022;81:e38.
2 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.
3 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.
4 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;79:e76.
5 Simpson EH. The interpretation of interaction in contingency tables. *J R Stat Soc Series B* 1951;13:238–41.
6 Wagner CH. Simpson's paradox in real life. *The American Statistician* 1982;36:46–8.
7 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.
8 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 histocompatibilty complex region in the Japanese population. *Nat Genet* 2019;51:470–80.