

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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Handling editor Josef S Smolen

Contributors JG undertook the primary duties in writing and editing the manuscript. TZ, XLW and HC undertook the duties in checking the primary data. ZL oversaw the manuscript writing and edited the manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Provenance and peer review Commissioned; internally peer reviewed.

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To cite Guo J, Zhang T, Li X, *et al*. *Ann Rheum Dis* Epub ahead of print: [please include Day Month Year]. doi:10.1136/annrheumdis-2020-217064

Received 25 February 2020

Revised 22 April 2020

Accepted 22 April 2020



► <http://dx.doi.org/10.1136/annrheumdis-2020-217031>

Ann Rheum Dis 2020;0:1–2. doi:10.1136/annrheumdis-2020-217064

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