Response to: 'Corresponence on 'Shared epitope defines distinct associations of cigarette smoking with levels of anticitrullinated protein antibody and rheumatoid factor' by Ishikawa *et al*' by Regueiro and Gonzalez

We are pleased to welcome the correspondence by Regueiro and Gonzalez[1] on our work.[2]

As they wondered if cigarette smoking (CS) introduced a differential association between the two subsets of patients with shared epitope (SE) and high anticitrullinated cyclic peptide/protein antibody (ACPA) described in figure 3B of the original manuscript, we add further explanation as follows; as shown in figure 3B of the original manuscript, smokers at the time of disease onset (SaO) with SE alleles have higher (OR 3.10, 95% CI 1.82 to 5.11, $p=2.3\times10^{-5}$) than never smokers with SE alleles (OR 2.24, 95%CI 1.56 to 3.24, $p=1.5\times10^{-5}$), while SaO without SE alleles does not have significant risk of high ACPA levels (OR 0.49, 95%CI 0.16 to 1.53, p=0.22). This indicates that CS does not independently affect ACPA production, but rather interacts with SE alleles and further increases the risk. Furthermore, we presented the linear association between SaO with SE alleles and ACPA levels (not presence of high ACPA level) in online supplementary figure S5 of the original manuscript, where the β coefficient of SaO with SE alleles (0.85, 95%CI 0.50 to 1.20, $p=2.8\times10^{-6}$) is higher than that of never smokers with SE alleles (0.55, 95%CI 0.34 to 0.77, $p=6.8\times10^{-7}$) implicating the former group of patients have higher ACPA titres. Furthermore, SaO without SE did not show even a positive trend of an association. These data clearly show the interactive effect of CS and SE alleles on ACPA production in patients with rheumatoid arthritis (RA).

They also suggested that a conditional analysis of one autoantibody on the other autoantibody, or stratified analyses on the serologically defined subgroups could clarify the interpretation of the results regarding specific autoantibodies given the common concurrent presence of rheumatoid factor (RF) and ACPA. While high levels of one autoantibody independently associated with high levels of the other autoantibody, conditional analysis by each autoantibody in addition to SE alleles still shows the same effects of SaO (and SE alleles) on high ACPA and RF levels as seen in figure 3A of the original manuscript (figure 1). Stratifying the patients according to the level of each autoantibody, ACPA(+) without high RF and RF(+) without high ACPA, did not change the association patterns observed in Figure 3B of the original manuscript (figure 1B). Moreover, stratifying the patients by different serotypes, ACPA(+) RF(-) (excluding RF(+) subjects from ACPA(+) subjects) and RF(+) ACPA(-) (excluding ACPA(+) subjects from RF(+) subjects), still showed the same trends, but lost significance due to the limited numbers of patients in some subgroups (figure 1C). Taken together, these two additional analyses indicate that the associations among SE, CS and each autoantibody production were not confounded by the presence of the other autoantibody frequently found in patients with RA.

Accordingly, our data further show the distinctive effect of CS on the presence and levels of ACPA and RF in Japanese RA patients. Importantly, our findings well fit with the work by Hedström *et al*,[3] indicating that the distinctive effects of CS on ACPA and RF are not limited on a certain ethnic groups or specific SE epitope alleles. Indeed, we also showed that amino acid (AA) position 74 has the most significant effect on the presence and high levels of ACPA by the omnibus analyses as in figure 5 of the original manuscript, meaning that this AA position, instead of specific SE alleles, might be a critical driver for ACPA development and the effect of CS on ACPA levels might also be dependent on this AA position.

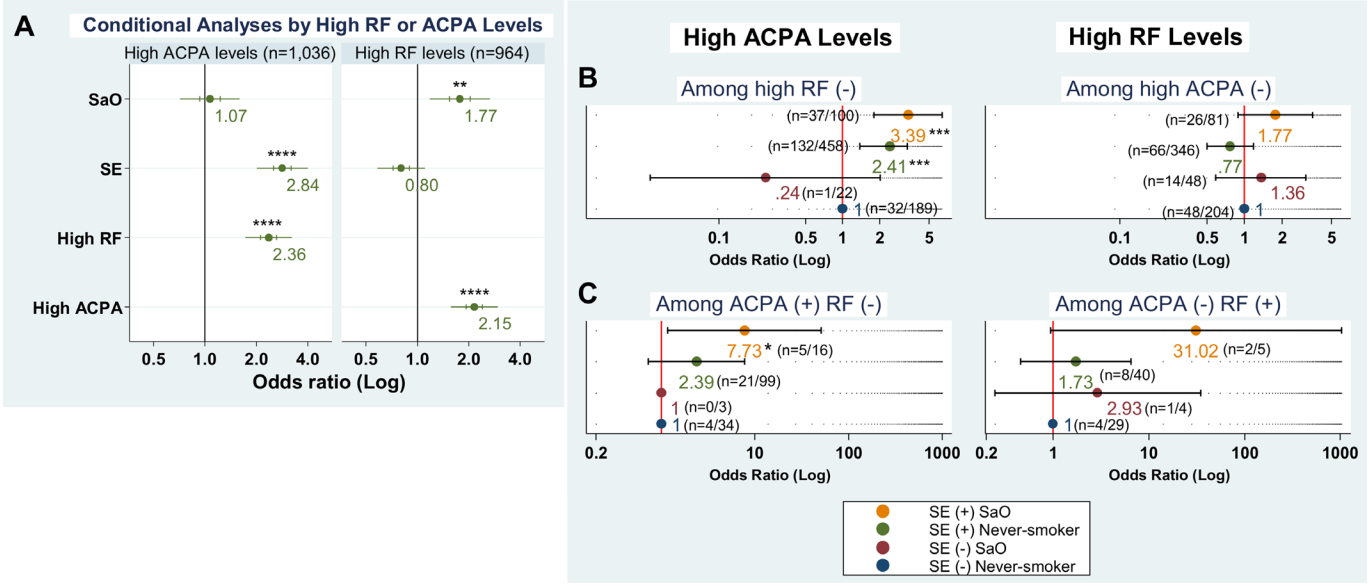

Figure 1 Cigarette smoking affects ACPA levels only in patients with SE alleles while cigarette smoking per se affects high RF levels regardless of SE allele status. (A) The association of smoking at the time of disease onset (SaO) with high ACPA or RF levels were evaluated conditioning on presence of SE alleles and high levels of RF or ACPA. ORs are indicated by dots and numbers, and 95% CIs are indicated by two-sided lines. (B, C) The associations of SaO with high ACPA or RF levels with or without SE alleles were evaluated referring never-smokers without SE alleles. Patients were stratified according to levels of autoantibodies (B; high ACPA without high RF or high RF without high ACPA) or serotypes (C; ACPA+RF or ACPA-RF+). ORs are indicated by dots and numbers, and 95% CIs are indicated by two-sided lines. The numbers of patients (case/total) are also indicated. High ACPA or RF: top quartile of ACPA or RF positive patients. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. ACPA, anticitrullinated cyclic peptide/protein antibody; RF, rheumatoid factor; SaO, smokers at the time of onset; SE, shared epitope.

The importance of this AA position for ACPA development was also reported by the studies on Caucasian populations[4 5] as well as Japanese populations,[6] further implicating a transethnic effect of this AA position on ACPA development. Further studies focusing on precise molecular mechanisms will be of particular interest.

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