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RAPID COMMUNICATION

The modifying influence of HLA class II DQB1*06:02 on the *Streptococcus* and clinical phenotype correlation among anti-Ro+ mothers of children with neonatal lupus

The genus *Streptococcus*, a gram-positive bacterium, contains species associated with immune activation at morbid outcomes, including scarlet fever, endocarditis, meningitis, and rheumatic fever. In both stool and saliva, there is a positive correlation between *Streptococcus* abundance and Sjogren's syndrome (SS) and systemic lupus erythematosus (SLE).[1,2] Interestingly, the HLA class II DQB1*06:02 allele is both associated with the risk of SLE[3] and plays putative roles in *Streptococcus* biology such as mimicry of bacterial antigens and related host antigens, an event that is associated with a loss of tolerance. We hypothesized that the *Streptococcus*—SLE association might be mediated by the presence of SLE-risk allele DQB1*06:02. Similarly, intercellular adhesion molecule-1 (ICAM-1), also known as CD54, and B-cell activating factor (BAFF), also known as tumor necrosis factor ligand superfamily member 13B (TNFSF13B), are elevated in the serum of SLE patients.[4] The serum protein levels of BAFF and ICAM1 may therefore further complicate the interplay among *Streptococcus* sp., DQB1*06:02, and SLE.

Anti-Ro+ mothers of children with neonatal lupus exhibit preclinical/early autoimmunity and are a population that may enable the identification of initiators of a first autoimmune response. The variation in *Streptococcus* relative abundance in anti-Ro+ mothers of children with neonatal lupus noted above was present in both oral and gut mucosa and observed when contrasting healthy controls (HC) and two groups of anti-Ro+ women spanning degrees of preclinical and clinical groups of SLE (*i.e.*, women who were asymptomatic or had undifferentiated autoimmune syndrome (Asym/UAS), and women with SS or SLE). The current study leverages anti-Ro+ mothers of neonatal lupus children from the Research Registry for Neonatal Lupus (RRNL)[5] to explore the hypothesized complex interplay among stool *Streptococcus* relative abundance, DQB1*06:02, and BAFF and ICAM1 levels in serum, and their potential role in SLE risk and progression from pre-clinical to clinical disease.

The anti-Ro+ mothers of children with neonatal lupus in the RRNL and the stool 16S microbiome sequencing and HLA genotyping assays were as previously described.[2] *Streptococcus* relative abundance was center log ratio (CLR) transformed, hereafter referred to as relative abundance, consistent with the compositional nature of 16S data. ICAM1 and BAFF were detected using the validated multiplex bead-based (xMAP) immunoassay in the serum of 39 anti-Ro+ positive women with a blood sample drawn near the time of stool specimen collection and who had DQB1*06:02 genotype data.

Streptococcus relative abundance varies as a combined function of both DQB1*06:02 status and the clinical state (Fig. 1, Panel A). Overall, *Streptococcus* relative abundance was higher in those women without the DQB1*06:02 allele. In those anti-Ro+ mothers with the DQB1*06:02 allele, *Streptococcus* relative abundance was lowest in controls and highest in SLE patients (HC < Asym/UAS < SLE; Fig. 1, Panel A). However, in those anti-Ro+ mothers without the DQB1*06:02 allele, there was no statistical difference in *Streptococcus* relative abundance across control, preclinical, and clinical groups (Fig. 1, Panel A; $P = 0.20$). Thus, these data suggest that DQB1*06:02 modifies the relationship between *Streptococcus* and clinical disease state for SLE in anti-Ro+ mothers of children with neonatal lupus (interaction $P = 0.022$).

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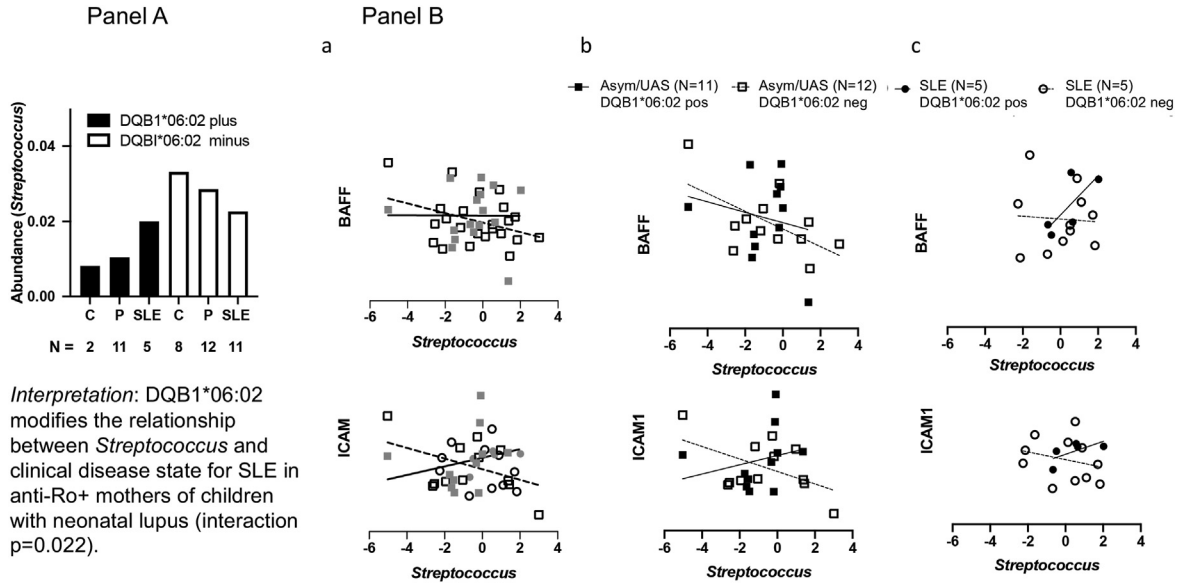

Figure 1 HLA DQB1*06:02 allele interaction with *Streptococcus* across clinical outcomes and key cytokines BAFF and ICAM1. **Panel A**: Illustration of the modifying effect of the presence of the DQB1*06:02 allele on the relationship between the genus *Streptococcus* relative abundance in stool and clinical status of anti-Ro+ mothers of children with neonatal lupus. DQB1*06:02 was selected because of the robust association with SLE in the literature and the allele demonstrated adequate frequency in our cohort for valid tests. Note in women with the DQB1*06:02 allele (Filled bars) in Panel A, the levels of *Streptococcus* were highest in SLE patients compared to Asym/UAS (the preclinical state, denoted P) and healthy controls (C). In contrast, in those women without the DQB1*06:02 allele (open bars), *Streptococcus* relative abundance does not differ statistically across the three clinical states. DQB1 *0602 modifies the relationship between S*treptococcus* and clinical disease state for SLE in anti-Ro+ mothers (Interaction $P = 0.022$). **Panel B** showed the associations of listed predictors *vs.* outcomes (cytokines and clinical group). Panel B: *Streptococcus* was negatively associated with ICAM1. The effect may be modified by the presence of the DQB1*0602 allele (**Panel B.a.*lower***, interaction $P = 0.026$). *Streptococcus* was negatively associated with BAFF but there was no evidence that DQB1*0602 modified this effect when adjusting for the clinical state (**Panel B.a.*upper***, interaction $P = 0.38$). Note, BAFF **(Panel B.c.*upper*)** in SLE showing a positive slope observed in the five SLE patients with the DQB1*0602 risk allele was different from other mothers ($P = 0.08$).

Streptococcus relative abundance also correlates with both BAFF and ICAM1 serum levels in anti-Ro+ mothers. In a linear regression model with ICAM1 as the outcome and adjusting for 16S batch processing and the clinical state ($n = 20$ Asym/UAS *vs.* $n = 19$ SLE) as covariates, *Streptococcus* was negatively associated with ICAM1 ($\beta = -55.04$, SE(β) = 22.92; $P = 0.022$). Here the effect of *Streptococcus* may be modified by the presence of the DQB1*06:02 allele (*i.e.*, *Streptococcus*-by-DQB1*06:02 interaction; $P = 0.026$) (Fig. 1, Panel B.a.*lower*). In a parallel analysis with BAFF as the outcome, *Streptococcus* was also negatively associated with BAFF ($\beta = -64.45$, SE(β) = 33.16; $P = 0.060$), but there was no evidence that DQB1*06:02 modified this effect (*i.e.*, no *Streptococcus*-by-DQB1*06:02 interaction; $P = 0.37$; Fig. 1, Panel B.a.*upper*). Note (Fig. 1, Panel B.c.*upper*), of BAFF in SLE, showing a positive slope observed in the five SLE patients with the DQB1*0602 risk allele was different from other mothers ($P = 0.08$).

Given the *Streptococcus,* BAFF, and ICAM1 associations, we tested whether *Streptococcus* was associated with the clinical state of the 39 anti-Ro+ mothers even after adjusting for ICAM and BAFF. Specifically, *Streptococcus* relative abundance was positively associated with SLE ($n = 22$ Asym/UAS *vs.* $n = 17$ SLE; OR = 3.81 per one standard deviation in *Streptococcus*, $P = 0.037$) after adjusting for batch, ICAM, and BAFF (Fig. 1, Panel A). A comparable effect was observed within each stratum when stratifying by the presence of the DQB1*06:02 allele and adjusting for batch, ICAM, and BAFF serum levels. Specifically, in the 16 mothers that had at least one DQB1*06:02 allele ($n = 11$ Asym/UAS *vs.* $n = 5$ SLE), the *Streptococcus* association remained, albeit the statistical significance was dampened due to reduced sample size ($P = 0.087$) (Fig. 1, Panel A). In the 23 anti-Ro+ mothers with no DQB1*06:02 alleles ($n = 11$ Asym/UAS *vs.* $n = 12$ SLE), the *Streptococcus* association similarly had dampened statistical significance due to reduced sample size ($P = 0.17$).

These data showing significant associations of *Streptococcus* and subjects with DQB1*0602 support a putative link between HLA and the loss of tolerance in anti-Ro+ mothers of neonatal lupus. In addition, these data show a bacterial interaction with an influence on BAFF and ICAM1, known lupus biomarkers.

The sample size in this study only allows the detection of large interaction effects. Also, a small sample size precludes an efficient adjustment for a range of confounders or complete multivariate modeling. Given the central role that genes within the HLA region have in the immune response, these data suggest that microbiome-disease paradigms will benefit from the active consideration of the HLA allele's potential to enhance or dampen the relationship of associated taxa with the disease. Evidence that the

SLE HLA risk allele confers an ordered clinical severity at the genus, *Streptococcus*, supports the hypothesis that *Streptococcus* is a risk factor of SLE with properties that include the recruitment of BAFF and ICAM1 as an inflammatory stimulus leading to an overactivation of the immune system in SLE.

Conflict of interests

The authors have no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.11.006.

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