Exploring the landscape of rheumatoid arthritis: piecing together risk factors and autoantibodies

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Letter

Genetic predisposition (HLA-SE) is associated with ACPA IgG variable domain glycosylation in the pre-disease phase of RA

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In addition to Fc glycans, Immunoglobulin G (IgG) can carry N-linked glycans in the variable domain. The abundant presence of disialylated variable domain glycans (VDGs) is a special feature of ACPA IgG and possibly other autoantibodies. The introduction of glycosylation sites is mediated by somatic hypermutation (SHM), a T-cell dependent process.[1] The high frequency of glycosylation sites does not correlate with the number of SHM, pointing towards a selective advantage of B cells expressing variable domain glycosylated ACPAs.[2] Previously, we observed that ACPA IgG VDGs are already present in the phase preceding the onset of RA and are predictive for disease development.[3] In addition, we provided first evidence that the human leukocyte antigen (HLA) "shared epitope" (SE) alleles, the most prominent genetic risk factor for ACPA-positive RA, are associated with the presence of VDGs on ACPA IgG pre-disease.[4] Hence, variable domain glycosylation could possibly explain the contribution of HLA-SE restricted T cells in the maturation of the ACPA response. Building upon these results, we now hypothesized that HLA-SE alleles may not be associated with ACPA-positivity as such, but with the specific presence of variable domain glycosylated ACPA IgG, a favorable factor for the development of this multifactorial disease.

To substantiate our hypothesis, we expanded the set of pre-symptomatic individuals (n = 228) and RA patients (n = 126) from Sweden and analyzed two additional cohorts comprising ACPA-positive Dutch subjects with arthralgia (n = 239) and ACPA-positive healthy Japanese individuals (n = 58) (Table S1). We determined the presence/percentage of ACPA IgG VDGs using liquid chromatography[5] and assessed associations with HLA-SE (supplementary materials and methods). In particular, we focused on the most prominent glycan peak (GP24) found on top of the variable domain,[1] which carries a bisecting N-acetylglucosamine and two terminal sialic acids (G2FBS2) (Figure 1A). ACPA IgG VDGs were, with a median of 58%, already abundantly expressed in healthy individuals (Table S1), in contrast to conventional IgG molecules that yield 12% of VDG.[6] VDG ($p = 0.047$) and GP24 ($p = 0.003$) were significantly higher in HLA-SE+ Dutch individuals with arthralgia compared to the HLA-SE-negative group (Figure 1B, Table S2 and S3). HLA-SE DR4+ (HLA-DRB1*04:01, *04:04, *04:05, *04:08 and *04:10 alleles) individuals showed the strongest increase in VDGs ($p = 0.009$) and GP24 ($p = 0.005$) compared to HLA-SE-negative subjects (Figure 1B). Even though, we observed a strong correlation between VDGs and ACPA-levels (Figure S1), we could not identify an association between ACPA-levels and HLA-SE ($p = 0.66$) (Table S4). Moreover, in line with our hypothesis, the association between HLA-SE and GP24 remained significant after correcting for ACPA-levels in a multivariable analysis (HLA-SE: $p = 0.03$; HLA-SE DR4+: $p = 0.07$) (Table S3), indicating that HLA-SE primarily associates with abundantly variable domain glycosylated ACPAs.

Interestingly, subjects with an "incomplete" VDG (lower than the median of 75%) (Table S1) were more prone to transition to RA, if they were HLA-SE DR4+ (HR: 2.74, p = 0.029) (Figure 1C). Conceivably, HLA-SE restricted T cells increase SHM and hence the formation of glycosylation-sites, impacting on a subsequent increased risk to develop disease.[3] Likewise, although underpowered and statistically not significant, VDGs and GP24 were numerically increased in the healthy ACPA-positive subjects from Japan, mainly in the HLA-SE DRB1*04:05+ group, the predominant HLA-SE alleles in this population (Figure 1D). The association between HLA-SE and increased VDG percentages was not present in the Swedish data-set, possibly because all subjects transition to RA (Table S5). However, the findings replicated our previous data, as HLA-SE alleles associated with the presence of ACPA IgG VDGs in the pre-RA phase, after correcting for ACPA-positivity (OR: 1.998, p = 0.040) (Table S7). No association was found between HLA-SE and ACPA in a reciprocal analysis (i.e. after correcting for the presence of VDG) (OR: 0.620, p = 0.254) (Table S8). Similar to our preceding analyses, this correlation was only found pre-disease as we could not identify a link between HLA-SE and VDGs in established disease (OR: 0.305, p = 0.269) (Table S9), likely because most ACPA IgG carry an abundant amount of VDGs by then (Table S1). Thus, in the phase preceding RA, HLA-SE alleles are associated with ACPAs harboring elevated amounts of VDGs. Additionally, HLA-SE+ individuals showed a significant increase in VDGs towards disease onset (matched paired analysis, Figures 1, E and F).

Hence, the data presented support a concept in which HLA-SE restricted T cells stimulate the introduction of glycosylation-sites in ACPA-expressing B cells, an event taking place before the development of ACPA-positive disease. HLA-SE can thus be considered as an "accelerating factor" causing the abundant expression of VDGs on ACPA IgG (Figure 1G). Our data also provide an explanation for why HLA-SE do not associate with ACPAs in healthy individuals[7, 8] as these are not yet abundantly glycosylated in their variable domains. The fact that all ACPA IgG are heavily glycosylated in established RA, explains why HLA-SE associate with ACPAs in this disease-stage and emphasizes the possibility that VDGs serve as an important "hit" involved in the unrestrained expansion of the RA-specific autoreactive B-cell response.

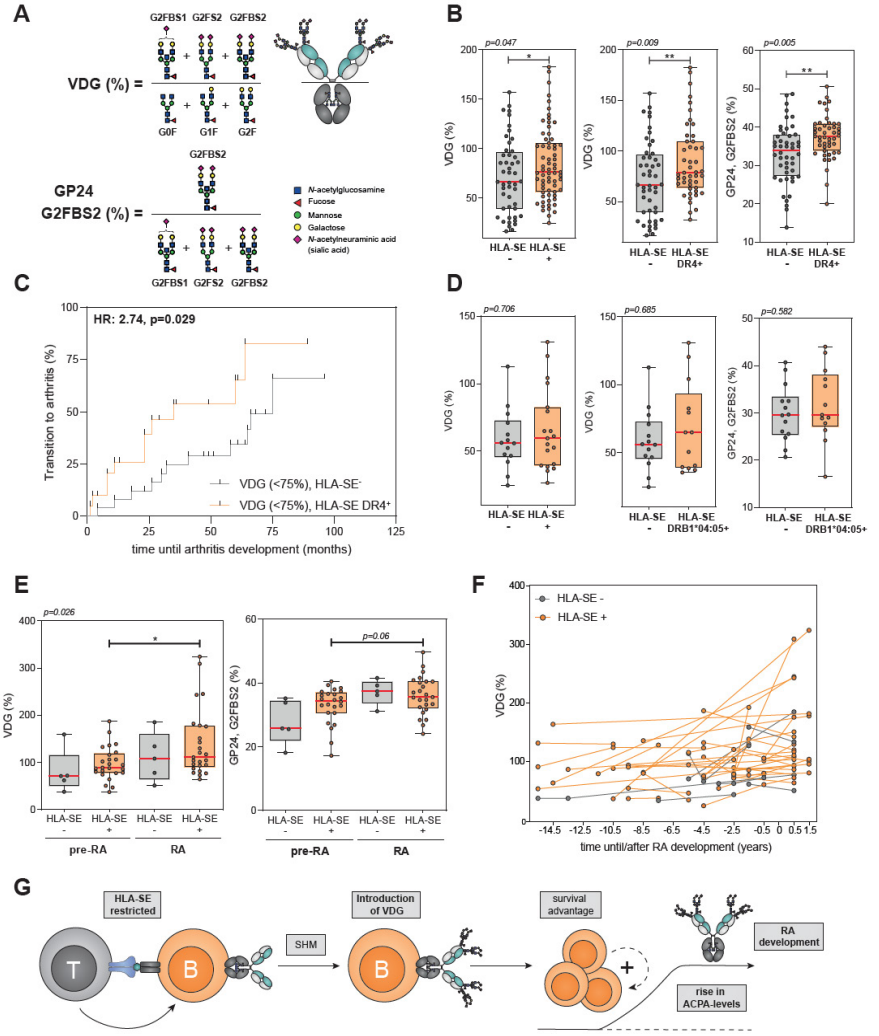

Figure 1. Percentage of ACPA IgG variable domain glycosylation (VDG) and glycan peak 24 (GP24, G2FBS2) in HLA-SE- and HLA-SE+ individuals. (A) Formulas to calculate the percentage of ACPA IgG VDG and the most common complex-type disialylated glycan peak found on top of the variable domain, GP24. ACPA IgG were captured, glycans released using PNGase F, 2-AA labelled, HILIC SPE purified and analysed using UHPLC. The formulas presented are based on the abundance of the liquid chromatography determined Fc-glycan traits G0F, G1F, G2F and VD glycan-traits G2FBS1, G2FS2 and G2FBS2. The respective glycans and their locations on the antibody molecule are schematically illustrated. Agalactosylated (G0), monogalactosylated (G1), digalactosylated (G2), fucose attached to the core GlcNAc (F), bisecting GlcNAc (B), monosialylated (S1), disialylated (S2). Blue square: N-acetylglucosamine (GlcNAc), green circle: mannose, yellow circle: galactose, red triangle: fucose, pink diamond: N-acetylneuraminic acid. (B) ACPA IgG+ individuals with arthralgia from the Netherlands (Amsterdam). Increased ACPA IgG VDG of HLA-SE+ (n = 67) compared to HLA-SE- (n = 48) individuals. Significantly higher ACPA IgG VDG and GP24 in HLA-SE DR4+ (n =

Figure 1. *Continued*
47) individuals. (C) ACPA IgG+ individuals with arthralgia from the Netherlands (Amsterdam) with a VDG < 75% (n = 49). ACPA IgG+ arthralgia individuals with a VDG lower than 75% are more prone to transition to disease and transition earlier, if they are HLA-SE DR4+ (HR: 2.74 (95% CI: 1.07 to 7.00), p-value: 0.029). (D) ACPA IgG+ symptom-free healthy individuals from Japan (Nagasaki). Statistically not significant trend for an increased percentage of ACPA IgG VDG and GP24 in HLA-SE+ (n = 19) particularly HLA-SE DRB1*04:05 (n = 13) healthy individuals compared to the HLA-SE- (n = 14) group. (E), (F). Matched pairs of samples from pre-symptomatic individuals and RA patients from Sweden (Umeå) (n = 59). HLA-SE+ individuals show a significant increase in their percentage ACPA IgG VDG and GP24 towards disease onset. HLA-SE+ pre-symptomatic individuals (n = 24) show already high VDG levels up to 15 years before RA-onset. (G). Graphical illustration of concluding hypothesis. HLA-SE restricted T cells give help to ACPA IgG-expressing B-cells, which results in SHM and the introduction of N-linked glycan sites, and consequently VDG (associations between HLA-SE and VDG). These ACPA IgG VDG+ B-cells expand leading to a rise in ACPA-levels and ultimately towards disease development. Mann Whitney U tests or linear regression analysis were performed between non-paired and Wilcoxon signed rank test between matched paired samples. The comparison of the survival curves was performed using a Mantel-Cox test. Significant differences are denoted by * or ** and the respective p-values are represented.

Supplementary material
Supplementary material available at:
https://www.sciencedirect.com/science/article/abs/pii/S0003496724204118?via%3Dihub

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Conflict of interest
H.U.S., T.W.J.H. and R.E.M.T. are mentioned inventors on a patent on ACPA IgG V-domain glycosylation.

Author contribution
TK: study concept and design, conducting experiments, aquisition of data, analysis and interpretation of the results, drafting and revising the manuscript, final approval of the manuscript. TJvW: study concept and design, statistical data analysis and interpretation of the results, drafting and revising the manuscript, final approval of the manuscript. AL and HK: statistical data analysis and interpretation of the results, critical revision and final approval of the manuscript. AK, MT, DvS, MW, TWJH and HUS: study concept and design, interpretation of the results, critical revision and final approval of the manuscript. DvdW, SR-D and REMT: study concept and design, interpretation of the results, drafting and revising the manuscript critically, final approval of the manuscript.

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