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A study of causal relationships between human IgG N-glycosylation traits and twelve associated diseases

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***Abstract* — N-glycosylation of IgG affects ligand binding, antigen recognition and modulates immune response. IgG N-glycome is altered in many pathological states, including cancers, autoimmune and inflammatory diseases. However, the causal relationships between diseases and IgG N-glycosylation traits remain enigmatic. In the current study we implement a Mendelian Randomization approach to study causal effect of IgG N-glycan traits on 12 diseases and vice versa. We have found limited genetic evidence that increased risk of systemic lupus erythematosus might lead to increased bisection of IgG N-glycans.**

Keywords* — *N-glycosylation, immunoglobulin G, Mendelian Randomization, inflammatory diseases, autoimmune diseases, systemic lupus erythematosus

Introduction

N-glycosylation is a post-translational modification, which impacts protein folding, stability, trafficking, ability to interact with other biomolecules, etc. The most abundant N-glycosylated antibody found in the blood plasma of healthy humans is immunoglobulin G (IgG), a major component of humoral immunity. Each IgG molecule has two conservative N-glycosylation sites in the fragment crystalizable region, and up to 20% of IgG molecules also exhibit N-glycans attached to the fragment antigen-binding domain. Majority of IgG N-glycans consist of a conserved core with two N-acetylglucosamine (GlcNAc) antennae, and may contain core-fucose, bisecting GlcNAc, and antennary galactosylation and sialylation. The structure of attached N-glycans affects the affinity of IgG to its ligands, antigen-binding and modulates immune response [1, 2]. IgG N-glycosylation profile is highly heritable [3], and a number of genetic variants showed association with IgG N-glycosylation features [4]. At the same time, N-glycosylation of IgG is altered in various physiological and pathological conditions, like ageing, cancer, inflammatory and autoimmune diseases [5].

It is still unclear, whether the lack or increase of some N-glycan structures on IgG is a risk factor for some diseases, or if it is the disease progression that leads to the changes in the IgG N-glycome. In this study we applied the two-sample Mendelian Randomization (MR) approach to investigate the causal relationships between IgG N-glycosylation traits and 12 inflammatory, autoimmune, cardiovascular and neurodegenerative diseases using summary statistics from publically available and in-house genome-wide association studies (GWAS).

Materials and Methods

Participating Cohorts

Diseases were included in the analysis based on the following criteria: 1) publically available GWAS summary statistics; 2) change of IgG N-glycosylation in the disease observed in a large-scale glycomic case-control study. Summary statistics for the IgG N-glycosylation traits were obtained from the studies of 8 cohorts of European ancestry: CROATIA-Korcula, CROATIA-Vis, ORCADES, TwinsUK, EGCUT, FINRISK, VIKINGS and CROATIA-Korcula2 [4, 6]. Following meta-analyses were performed: CROATIA-Korcula, CROATIA-Vis, ORCADES, TwinsUK (N=8090, further on referred to as the 8K cohort); 8K, EGCUT and FINRISK (N = 9190, 9K cohort); EGCUT, FINRISK, VIKINGS and CROATIA-Korcula2 (N=3147, 3K cohort).

Two-sample MR Analysis

Two-sample MR was performed using "TwoSampleMR" R package [7]. The causal effect estimate was obtained as an inverse variance weighted meta-analysis of ratios of exposure effect size and outcome effect size for each of the instruments. We performed three rounds of the analysis: in the discovery round we were testing causal effects in all possible pairs of IgG N-glycosylation traits and diseases in both directions; in the following two rounds of sensitivity analysis we were trying to replicate the causal effect of systemic lupus erythematosus (SLE) on bisection of IgG N-glycans (IgG_B) observed in the discovery round.

Discovery Round

To estimate causal effects of IgG N-glycosylation traits on diseases, we selected genetic variants that were 1) significantly associated with IgG N-glycosylation in both [4,6]; 2) the association was replicated in the 9K cohort (p-value < 5e-08). As exposure we used only summary statistics for the 56 traits that had a least 2 significant instruments. As outcomes we used publically available summary statistics for the 12 diseases. To test the causal effect of diseases on IgG N-glycosylation, as instruments we used independent genetic variants with associated p-value < 5e-08 from corresponding disease summary statistics. As outcomes we used summary statistics of 86 IgG N-glycosylation traits in the 8K cohort.

Sensitivity Analysis of the SLE Effect on IgG_B

To perform sensitivity analysis for the causal effect of SLE on IgG_B, as instruments we used a refined set of 36 genetic variants associated with SLE [8], excluding outliers identified

with the MRPRESSO package for R [9]. As outcomes we have used summary statistics for IgG_B in the 8K cohort individuals in the first round of sensitivity analysis, and in the 3K cohort for the second round.

Results

We performed MR analysis in both directions, testing causal effects of IgG N-glycosylation traits on the risk of 12 diseases and *vice versa*. On the discovery stage, a signal related to the casual link of SLE on bisection of IgG N-glycans (IgG_B) survived after the correction on multiple testing. The causal effect was estimated as 0.131 standard deviation units of IgG_B per log odds units of SLE (p value = 2.24e-09). To confirm the detected casual signal we performed two rounds of sensitivity analyses. In the first round, where a refined set of genetic variants was used as instruments, the causal effect was confirmed with p-value of 1.24e-05. However, in the second round of sensitivity analysis, where the summary statistics for IgG_B from the 3K cohort were used, the causal effect of SLE on IgG_B was completely lost. The results of all MR rounds for SLE as exposure on IgG_B as outcome are shown in Table 1. In conclusion, the observed genetic support for causal relationships between IgG N-glycosylation is limited and further studies of the regulation of IgG N-glycosylation are required.

TABLE 1 – CAUSAL EFFECT OF SLE ON BISECTION OF IGG N-GLYCANS ESTIMATED BY MENDELIAN RANDOMIZATION

Analysis round	# of SNPs	SNP selection	beta	se	p-value
Discovery	33	Clumping	0.13	0.022	2.2e-09
Sensitivity 1	36	Literature	0.12	0.028	1.2e-05
Sensitivity 2	35	Literature	-0.03	0.045	0.49

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