Genetically downregulated interleukin-6 signaling is associated with a favorable cardiometabolic profile: a phenome-wide association study

Running title: *Georgakis et al; Phenotypic outcomes of downregulated IL6 signaling*

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Interleukin-6 (IL6) signaling is a key inflammatory pathway involved in activation and regulation of immune responses, tissue regeneration, and metabolism.[1] While IL6-receptor (IL6R) inhibitors are already in use for the treatment of autoimmune diseases,[2] accumulating evidence supports a broader role of IL6 signaling in human disease.[2,3] Still, it remains unknown whether IL6R blockade could be effectively repurposed for the treatment or prevention of diseases beyond current indications.

We recently identified 7 genetic variants in the *IL6R* locus showing similar effects on upstream (soluble IL6R and IL6) and downstream (C-reactive protein [CRP] and fibrinogen) molecules in the IL6 signaling cascade as those derived from clinical trials for IL6R inhibitors.[4] Here, to systematically explore potential repurposing opportunities and unknown side-effects associated with IL6R blockade, we used these variants as proxies of IL6 signaling downregulation and examined widespread effects in a phenome-wide association study (PheWAS). Specifically, we analyzed 1,428 clinical outcomes in up to 339,256 White British individuals from the UK Biobank study and validated the identified signals in a meta-analysis with the Penn Medicine (10,244 individuals) and the BioMe (9,054 individuals) Biobanks of European American individuals. We further analyzed 366 disease-related biomarkers including hematological, biochemical, metabolomic, inflammatory, immunological, hemodynamic, and anthropometric traits in the UK Biobank and phenotype-specific genetic consortia. We pooled the SNP-specific effects using Mendelian randomization (MR) analyses scaled to the effects of tocilizumab, an IL6R-targeting monoclonal antibody. A detailed description of methods and summary statistics for the presented analyses are provided elsewhere.[5] Supporting data for the UK Biobank analyses are available online (Supplementary data),[5] whereas supporting data from Penn Medicine and BioMe Biobanks are available upon request to the principal investigators of the study. All participants provided informed consent and all studies obtained IRB approval as detailed elsewhere.[5]

There were 35 clinical outcomes reaching statistical significance (FDR<0.05, $p<1.7\times10^{-3}$) in the primary inverse-variance-weighted MR analyses; 33 of them showed no evidence of heterogeneity ($p>0.10$) while exhibiting consistent associations (same direction, $p<0.05$) in

sensitivity analyses (weighted-median MR, analyses restricted to 3 SNPs within *IL6R*). In the meta-analysis of the UK Biobank with the PMBB and BioMe Biobanks, 16 of the 24 outcomes with sufficient statistical power for validation remained significant ($p<1.7x10^{-3}$; **Figure 1A**). There were significant associations of genetically downregulated IL6 signaling with lower risk of several atherosclerotic phenotypes including ischemic heart disease (OR: 0.84, 95%CI: 0.77-0.90) and abdominal aortic aneurysm (OR: 0.44, 95%CI: 0.29-0.67), as well as with lower risk of type 2 diabetes (OR: 0.80, 95%CI: 0.73-0.88). Conversely, we found associations of genetically downregulated IL6 signaling with higher risk of cellulitis and abscess of arm/hand, urinary tract infections, other disorders of urethra and urinary tract, female infertility, unspecified erythematous conditions, and atopic dermatitis**.**

In the analyses for biomarkers, 25 associations reached statistical significance (FDR<0.05, $p<1.8x10^{-4}$). Of them, 17 were consistent in sensitivity analyses and did not show significant heterogeneity (**Figure 1B**). Aside from the expected associations with higher CRP and lower IL6 levels,[4] we found associations with higher hemoglobin concentration and related traits, as well as higher monocyte count and percentage and lower granulocyte percentage. Furthermore, genetically downregulated IL6 signaling was associated with lower HbA1c. Among serum lipids and metabolites, genetically downregulated IL6 signaling was associated with higher total and HDL cholesterol. There were also significant associations with lower cystatin C, and higher levels of IL4.

Taken together, genetic downregulation of IL6 signaling was associated with (i) lower risk of atherosclerotic vascular phenotypes (coronary artery disease and abdominal aortic aneurysm), (ii) lower HbA1c and lower risk of type 2 diabetes, (iii) increases in total and HDL cholesterol levels, (iv) higher risk of neutropenia and skin and urinary tract infections, (v) higher risk of atopic phenotypes and higher levels of the pro-allergic cytokine IL4, and (vi) increases in hemoglobin and related phenotypes and monocyte counts.

Our study has limitations. First, IL6 signaling is a complex cascade with a classical component (exerted through membrane-bound IL6R expressed in limited tissues) and a

trans-signaling component (exerted through the more widely expressed soluble IL6R).[1] Disentangling these components goes beyond the limits of MR. Second, MR assesses the effects of lifetime downregulated IL6 signaling, which might differ from a shorter pharmacological inhibition with IL6R blockade. Third, the sample sizes of the validation cohorts in this study were rather small and did not offer sufficient power to explore the robustness of all signals that came up in the discovery cohort. Fourth, our results were solely based on individuals of European origin and might thus not apply to other ethnicities. Fifth, we proxied IL6 signaling using CRP and other upstream and downstream molecules of the IL6 signaling cascade; yet, the exact cellular and molecular significance of each variant remains unknown.

In conclusion, genetic IL6 signaling downregulation associates with a lower risk of atherosclerotic outcomes and a more favorable cardiometabolic profile including lower risks of type 2 diabetes and hyperglycaemia and higher HDL cholesterol levels. As such, our findings further highlight the potential of repurposing IL6R blockade as a strategy for lowering vascular risk. These effects should be further explored in clinical trials and weighted against the side-effects of IL6-targeting approaches.

Conflict of Interest Disclosures: Dr Gill is employed part-time by Novo Nordisk. The other authors have no conflicts of interest to disclose.

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Figure 1. Results of the phenome-wide association study (PheWAS) (A) for clinical outcomes in the UK Biobank and validation in PMBB and BioMe Biobanks, and (B) for biomarkers.

Shown are the results from the inverse-variance weighted Mendelian randomization analyses in the UK Biobank. The x-axes correspond to the logarithms of the p-values derived from these analyses. The red lines correspond to the statistical significance level (FDR<0.05). In panel (A) we present results from the UK Biobank and outcomes surviving all significance criteria in the UK Biobank analyses are labeled by name; outcomes that were further validated in the meta-analysis of UK Biobank with PMBB and BioMe biobanks are labeled in black, whereas those for which validation was unfeasible due to a low number of cases in both validation cohorts are labeled in grey. In panel (B), solid circles correspond to outcomes that survived all our significance criteria and open circles correspond to outcomes that showed significant associations while there was significant heterogeneity between the effects of the individual variants.