Interleukin-6 Signaling Effects on Ischemic Stroke and other Cardiovascular Outcomes: A Mendelian Randomization Study

Running title: *Georgakis et al.; IL-6 signaling and cardiovascular disease*

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Nonstandard Abbreviations and Acronyms

CRP	C-reactive protein
GWAS	Genome-wide association study
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IL-6R	Interleukin-6 receptor
MR	Mendelian randomization
SNP	Single nucleotide polymorphism

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Downregulation of interleukin (IL-6) signaling has been proposed as a strategy for lowering cardiovascular risk. Secondary analyses from CANTOS demonstrated that the therapeutic benefit of interleukin-1 (IL-1β) inhibition on cardiovascular prevention was associated with the reduction of IL-6 levels and that the residual cardiovascular risk was proportional to post-treatment IL-6 levels.[1] Moreover, Mendelian randomization (MR) analyses showed variation in IL-6 receptor gene (*IL6R*) to be associated with risk of coronary artery disease.[2] Thus, directly interfering with IL-6 signaling might lower cardiovascular risk beyond IL-1β inhibition. Whether such an approach would be effective for ischemic stroke and other cardiovascular outcomes (aortic aneurysm, carotid plaque, peripheral artery disease, atrial fibrillation, heart failure, thrombotic phenotypes) remains unknown.

Here, we identified genetic proxies for IL-6R-mediated downregulation of IL-6 signaling as variants within a region 300 kB 5' to 300 kB 3' to *IL6R* that were associated with lower C-reactive protein (CRP) levels. CRP is a well-established downstream molecule of IL-6 signaling

and a clinically useful biomarker for assessing residual inflammatory cardiovascular risk. Variants were derived from a genome-wide association study (GWAS) of 204,402 European individuals ($p<5x10^{-8}$; clumped at $r^2<0.1$). We identified 7 SNPs that served as instruments for downregulated IL-6 signaling (3 situated within *IL6R*). In conditional GCTA-COJO analyses[3] adjusting for the lead SNP (rs2228145), *p*-values for all SNPs were <0.05 (for SNPs within *IL6R* <0.0083-Bonferroni-corrected threshold) indicating independent effects on CRP levels. F-statistics ranged from 81 to 764. To validate these instruments, we explored associations of genetically downregulated IL-6 signaling with circulating upstream regulators (IL-6, soluble IL-6R) and downstream molecules (fibrinogen) of the IL-6 signaling pathway. In accordance with trials testing tocilizumab versus placebo,[2] genetically downregulated IL-6 signaling was associated with higher circulating IL-6 and soluble IL-6R levels and lower fibrinogen levels (**Figure A**).

Two-sample inverse-variance weighted MR analyses showed genetically downregulated IL-6 signaling to be associated with lower risks of ischemic stroke (MEGASTROKE: 34,217 cases, 404,630 controls) and coronary artery disease (CARDIoGRAMplusC4D: 60,801 cases, 123,504 controls) (**Figures B-C**). We further found associations with lower risks of large artery and small vessel stroke, but not cardioembolic stroke (**Figure D)**. Alternative MR approaches (weighted median, contamination-mixture, MR-PRESSO) and sensitivity analyses restricted to the variants within *IL6R* all showed consistent association estimates.

MR analyses revealed no significant associations between genetically determined CRP and ischemic stroke or its subtypes independently of whether we used all variants associated with CRP (187 SNPs) or SNPs at the *CRP* locus (24 SNPs). Furthermore, in permutations of MR analyses[4] randomly selecting 7 of the 187 SNPs associated with CRP, the effects of the 7 SNPs

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selected as instruments for downregulated IL-6 signaling on ischemic stroke and its subtypes were consistently located below the lowest 5th percentile of the respective distributions (**Figure E**). Thus, the observed effects were independent of the effects of CRP.

Finally, we expanded the analyses to other cardiovascular outcomes in the UK Biobank (321,406 individuals) and phenotype-specific GWAS datasets. Genetically downregulated IL-6 signaling was significantly associated with lower risks of myocardial infarction and aortic aneurysm. We further found suggestive associations (p<0.05) with atrial fibrillation and carotid plaque (**Figure F**). Again, these associations were independent of CRP levels.

Our results strongly support the candidacy of IL-6 signaling as a target for vascular prevention over and beyond previous data. CANTOS targeted IL-1β rather than IL-6R thus providing only indirect evidence for a benefit of interfering with IL-6 signaling.[1] Also, CANTOS explored a combined endpoint rather than individual cardiovascular outcomes. Regarding stroke, there was a 7% reduction in incident events in the IL-1β arm, which did not reach statistical significance, and data on stroke subtypes were not available.[5] Our MR results provide evidence for directionally consistent effects of IL-6 signaling on ischemic stroke and other cardiovascular outcomes and offer a solid basis for future trials exploring the benefit of pharmacological IL-6R inhibition for these phenotypes.

Our results are in broad agreement with a recent MR study using the same data sources but a different approach to explore the effects of IL-6 signaling on cardiovascular outcomes.[6] While that study used plasma levels of soluble IL-6R to proxy the effects of IL-6 signaling, we used CRP levels, which might explain some discrepancies in the results. We did not select variants based on their effects on IL-6 or soluble IL-6R, because they are upstream regulators of IL-6 signaling and variants increasing their levels could also upregulate the pathway. Still, IL-6

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signaling is complex with a classical and a trans-signaling component and disentangling the two sub-pathways goes beyond the limitations of MR.

In conclusion, this study provides evidence for a causal effect of IL-6 signaling on ischemic stroke, particularly large artery and small vessel stroke, as well as a range of cardiovascular phenotypes. IL-6R blockade might represent a valid therapeutic target for lowering cardiovascular risk and should thus be further investigated in clinical trials.

All data related to the effects of these variants on specific outcomes are publicly available as summary statistics from the respective sources. Data for outcomes derived from the UK Biobank are available after submission of a research proposal. All the data are also available from the corresponding authors upon reasonable request. All individual studies had obtained ethical approval by the appropriate institutional review committees, as described in the original publications.

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Disclosures: None

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Figure Legend:

Figure: Genetic proxies for downregulated IL-6 signaling and its effects on ischemic stroke and other cardiovascular outcomes in Mendelian randomization analyses. (A) Effects of pharmacological inhibition of IL-6R (tocilizumab 8 mg/kg vs. placebo for 8 to 24 weeks) and of genetic downregulation of IL-6 signaling on IL-6 (N_{trials}=1,446; $N_{genetics}$=8,293), sIL-6R (N_{trials}=1,465; $N_{genetics}$=3,301), and Fg levels (N_{trials}=1,108; $N_{genetics}$=120,246). (B) Genetically downregulated IL-6 signaling in association with ischemic stroke and coronary artery disease as derived from IVW analyses using the full set of 7 SNPs as instruments or the 3 SNPs located within the *IL6R* gene. (C) SNP-specific effects of the associations with ischemic stroke. (D) Genetically downregulated IL-6 signaling and ischemic stroke subtypes. (E) Distributions of the effects of 7 randomly selected CRP-decreasing SNPs on risk of ischemic stroke and the position of the effect of the IL-6 signaling downregulating SNPs included in our analyses. (F) Genetically downregulated IL-6 signaling with other cardiovascular outcomes. Effect sizes for genetically downregulated IL-6 signaling are scaled to the CRP-decreasing effects of tocilizumab (8 mg/kg). Statistical significance thresholds are set at $p<0.05/3=0.017$ for the 3 ischemic stroke subtypes, and at $p<0.05/9=0.0055$ for the 9 cardiovascular outcomes. Associations showing p-values <0.05 are considered suggestive. IL-6, interleukin 6; sIL-6R, soluble IL-6 receptor; Fg, fibrinogen; IVW, inverse-variance weighted; SNP, single nucleotide polymorphism; CRP, C-reactive protein. SMD, standardized mean difference.

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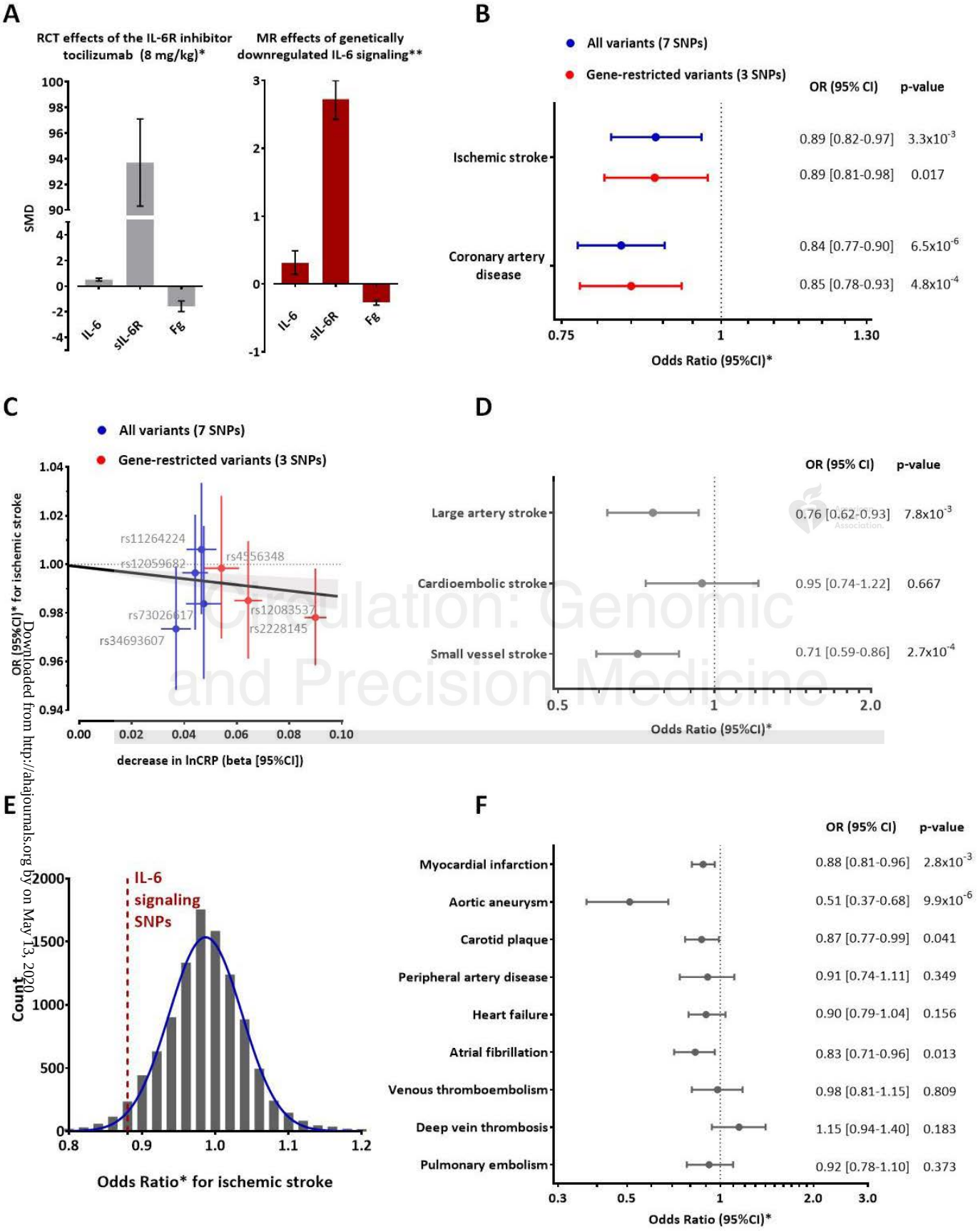

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