"C1-inhibitor levels and venous thromboembolism: results from a Mendelian randomization study": Reply

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Dear Editor,

We thank Grover et al. for their interest in our Mendelian randomization (MR) study where we did not find evidence to support a causal relation between genetically proxied C1-inhibitor levels and venous thromboembolism [1].

Grover et al. raise the concern that the 8 single nucleotide polymorphisms (SNPs) identified are insufficiently able to explain the observed variance in C1-inhibitor levels. While the use of common genetic variants results in a more subtle effect on protein levels than their rare counterparts, proportion of variance explained is merely important in the context of the required sample sizes for the outcome investigated. In fact, 10% due to 8 SNPs is quite significant, considering the fact that – for example – the first 97 loci that were identified for Body Mass Index (BMI) only accounted for 2.7 % of BMI variation [2]. Another influential MR paper investigating the effect of blood lipids on coronary heart disease showed that a 42-SNP (R^2 = 4.3) and a restricted 19-SNP score (R^2 = 3.38) for LDL-cholesterol were both significantly associated with coronary heart disease (in a cohort with 62 199 individuals) [3]. The smaller proportion of variance explained indeed requires larger datasets to provide sufficient power. This can be readily understood by noting that the R-squared is proportional to the p-value, where for a fixed effect (i.e. odds ratio) the p-value decreases with increasing sample size. Moreover, if the instruments would explain a more substantial amount of variability in the exposure this would imply the results would closely mirror that of an observational analysis regressing venous thromboembolism on circulating C1-inhibitor values, which are of course subject to possible confounding bias and reverse causation [4]. Hence, a large explained variability, e.g. 80%, would counteract the benefits of MR completely. This specific comment by Grover et al. illustrates a fundamental misunderstanding of the statistical principles underlying MR, where the aim is not to fully or comprehensively predict the exposure, but rather to identify potential causal exposure effects using genetic instrumental variables which are less susceptible to the aforementioned sources of bias.

We acknowledge there is a potential risk of cross-linking associated with the SomaScan aptamer assay. However, when Ferkinstad et al. compared the SomaScan method with the antibody-based Olink in a small subset of patients and proteins, they found a median correlation of 0.76, indicating good correspondence [5]. Another study by Sun et al. cross-referenced 163 somamers with Olink and found strongly-correlated effect size estimates (r=0.83) [6]. While cross-referencing remains an issue, it is of note that many somamers that do bind to homologous proteins bind to alternative forms of the same protein [7]. Our colleagues already referenced to a paper confirming the specificity of the C1-inhibitor binding aptamer [8].

As Grover et al. pointed out, Ferkinstad and colleagues reported an association between C1-inhibitor levels and myocardial infarction and coronary artery disease [5]. Besides the notion that these forms of arterial thrombosis form through a different underlying pathophysiology, this reported association between the level of circulating protein and disease is not based on Mendelian randomization. Consequently, as the authors recognize, the reported associations do not necessarily represent causal relationships where C1-inhibitor levels influence the risk of disease. C1-inhibitor levels could also be a consequence of disease or correlate with a disease risk factor without being a cause or consequence. While we agree that these findings do not contradict a potential thromboprotective role of C1-inhibitor, we urge for caution in interpreting them as supporting such a role.

Grover et al. further argue there is no firm association between C1-inhibitor administration and venous thromboembolic risk. We agree that the therapy itself does not pose a risk for venous thrombosis, but the route of administration requires (peripherally or centrally inserted) venous catheters, thereby increasing the risk of thrombotic occlusions. Thus, the aforementioned retrospective cohort study, which particularly reported an increased incidence of 'other VTE' (excluding deep venous thrombosis and pulmonary embolism) in patients with hereditary angioedema, could have been confounded by the utilisation of venous catheters [9].

We acknowledge that our study is limited by the absence of adjudication of venous thromboembolic events in the FinnGen cohort and UK Biobank. We emphasize that while our study could not reject the null hypothesis that C1-inhibitor levels are not causally associated with venous thromboembolism, this does not imply that the null hypothesis is necessarily correct. Instead, it highlights the need for further critical investigation of the existing evidence.

In their nested case-control study in the Tromsø cohort, Grover et al*.* recently reported a significant association between plasma C1-inhibitor levels and incident venous thromboembolism risk [10]. To their credit the authors utilized untargeted proteomics (similar to our analysis) and attempted to replicate their findings using an C1-inhibitor levels enzyme-immunoassays (EIA), representing a well calibrated measurement of protein concentration. The analysis of the untargeted C1-inhibitor levels indeed suggests a biologically plausible association where people with in the highest C1-inhibitor quartile have a smaller risk of VTE. Analysis of the EIA C1-inhibitor concentration instead reveals a more difficult to fathom pattern where people in quartile 2 (186-211 µg/mL) and quartile 4 (≥248 µg/mL) are equally protected against VTE: OR 0.71 (95%CI 0.50; 0.99) and OR 0.68 (95%CI 0.49; 0.96) for quartile 2 and quartile 4 compared to quartile 1, respectively. While potential non-linear associations are of course plausible, it is remarkable that people well within the normal range of C1-inhibitor concentration have the same VTE risk as people with a much higher C1-inhibitor concentration. Reverting back to their untargeted C1-inhibitor value, the authors attempt to find evidence for a potential non-linear association with VTE risk using spline transformation

(Supplementary Figure S2), but instead find strong support for a linear association, and while they do not present a p-value for this analysis, the confidence interval predominantly includes a neutral OR of 1, suggesting the association with VTE is at best nominal but probably non-significant. Additionally, given that these analyses are subject to confounding bias, it is worth noting that the authors did not account for established risk factors such as oral contraceptive usage and history of cardiovascular disease, potentially explaining their results. The notion that the authors were unable to find SNPs associated with C1-inhibitor levels in 707 patients (a cohort 50 times smaller than the Icelandic cohort used in our study) underscores the necessity of utilizing adequately large genome-wide association studies to detect such associations.

We fully agree with Grover et al. that the potential association between C1-inhibitor levels and venous thromboembolism is insufficiently understood. While our MR study did not support a causal association between C1-inhibitor levels and VTE, we of course recognize the pathophysiological rational supposing a causal relationship. We therefore wish to emphasise that our analysis, and lack of observed association, should not be misconstrued as strongly confirming an absence of effect. Rather our analysis simply suggests a need for prospectively designed studies incorporating accurate adjudication of venous thromboembolism events, comprehensive adjustment for all potential confounding factors, exploration of potential non-linear associations, with synergistic consideration of all sources of evidence.

Addendum

A.J. Cupido and R.S. Petersen wrote the manuscript. All authors contributed to the article and approved of the submitted version.

The authors declare no conflicts of interest.

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