Brief Report

Genetically Determined FXI (Factor XI) Levels and Risk of Stroke

Dipender Gill, MD; Marios K. Georgakis, MD; Mike Laffan, MD, PhD; Maria Sabater-Lleal, PhD; Rainer Malik, PhD; Ioanna Tzoulaki, PhD; Roland Veltkamp, MD*; Abbas Dehghan, MD, PhD*

Background and Purpose—FXI (factor XI) is involved in thrombus propagation and stabilization. It is unknown whether lower FXI levels have a protective effect on risk of ischemic stroke (IS) or myocardial infarction. This study investigated the effect of genetically determined FXI levels on risk of IS, myocardial infarction, and intracerebral hemorrhage.

Methods—Two-sample Mendelian randomization analysis was performed. Instruments and genetic association estimates for FXI levels were obtained from a genome-wide association study of 16 169 individuals. Genetic association estimates for IS and its etiological subtypes were obtained from a study of 16 851 cases and 32 473 controls. For myocardial infarction, estimates were obtained from a study of 43 676 cases and 123 504 controls and for intracerebral hemorrhage from a study of 1545 cases and 1481 controls.

Results—After applying a Bonferroni correction for multiple testing, the Mendelian randomization analysis supported a causal effect of higher, genetically determined FXI levels on risk of any IS (odds ratio [OR] per 1-unit increase in natural logarithm-transformed FXI levels, 2.54; 95% CI, 1.68–3.84; $P=1\times10^{-5}$) but not myocardial infarction (OR, 1.01; 95% CI, 0.76–1.34; $P=0.94$) or intracerebral hemorrhage (OR, 1.81; 95% CI, 0.44–7.38; $P=0.41$). Examining IS subtypes, the main results supported an effect of higher, genetically determined FXI levels on risk of cardioembolism (OR, 4.23; 95% CI, 1.94–9.19; $P=3\times10^{-4}$) and IS of undetermined cause (OR, 3.44; 95% CI, 1.79–6.60; $P=2\times10^{-4}$) but not large artery atherosclerosis (OR, 2.73; 95% CI, 1.15–6.45; $P=0.02$) or small artery occlusion (OR, 1.19; 95% CI, 0.50–2.82; $P=0.69$). However, the statistically significant result for IS of undetermined cause was not replicated in all sensitivity analyses.

Conclusions—We find Mendelian randomization evidence supporting FXI as a possible target to reduce risk of the cardioembolic subtype of IS. **(*Stroke*. 2018;49:2761-2763. DOI: 10.1161/STROKEAHA.118.022792.)**

Key Words: cardiovascular diseases ■ risk ■ stroke

FXI (factor XI) is an enzyme that is involved in thrombus propagation and stabilization. An observational study has suggested that individuals with FXI deficiency have reduced incidence of ischemic stroke (IS).[1] Furthermore, reduction of FXI levels was effective for preventing postoperative venous thromboembolism in patients undergoing total knee arthroplasty, without any apparent increase in bleeding complications as compared with standard care.[2]

The Mendelian randomization (MR) technique uses randomly allocated genetic variants that are related to an exposure as instruments to investigate the effect of varying that exposure.[3] To avoid bias, the genetic variants selected as instruments should only affect the outcome through the exposure and not by some pleiotropic pathway. To this end, genetic variants related to FXI levels that are located at the *F11* gene make viable instruments for use in MR.

We perform MR analyses using such instruments for FXI levels to investigate the effect of genetically determined FXI levels on risk of IS, IS etiological subtypes, and myocardial infarction (MI). To explore whether higher, genetically determined FXI levels cause increased risk of bleeding, we also examined associations with intracerebral hemorrhage (ICH).

Methods

The data used in this study are available from the corresponding author on reasonable request. Instruments and genetic association estimates for FXI were obtained from a genome-wide association study of natural logarithm-transformed FXI levels in 16 169 European individuals.[4] Instruments for the main analysis were selected as single-nucleotide polymorphisms (SNPs) within 100 kb of the *F11* gene (ie, chromosome 4 position 187,087,099-187,310,835 on GRCh37/hg19) that had genome-wide significant associations with FXI levels and were in low linkage disequilibrium with $r^2<0.001$.[4] Details

Received July 9, 2018; final revision received August 9, 2018; accepted August 22, 2018.

From the Department of Biostatistics and Epidemiology, School of Public Health (D.G., I.T., A.D.), Department of Stroke Medicine (D.G., R.V.), Centre for Haematology (M.L.), and MRC-PHE Centre for Environment, School of Public Health (I.T., A.D.), Imperial College London, United Kingdom; Institute for Stroke and Dementia Research, University Hospital of Ludwig-Maximilians-University, Munich, Germany (M.K.G., R.M.); Unit of Genomics of Complex Diseases, Institut d'Investigació Biomèdica Sant Pau, Barcelona, Spain (M.S.-L.); Department of Medicine Solna, Cardiovascular Medicine Unit, Centre for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden (M.S.-L.); Department of Hygiene and Epidemiology, University of Ioannina Medical School, Greece (I.T.); Department of Neurology, University of Heidelberg, Germany (R.V.); and Department of Neurology, Alfried Krupp Hospital, Essen, Germany (R.V.).

*Drs Veltkamp and Dehghan contributed equally.

The online-only Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/STROKEAHA.118.022792.

Correspondence to Dipender Gill, MD, Department of Biostatistics and Epidemiology, School of Public Health, St Mary's Hospital, Imperial College London, Medical School Bldg, Norfolk Pl W2 1PG, United Kingdom. Email dipender.gill@imperial.ac.uk

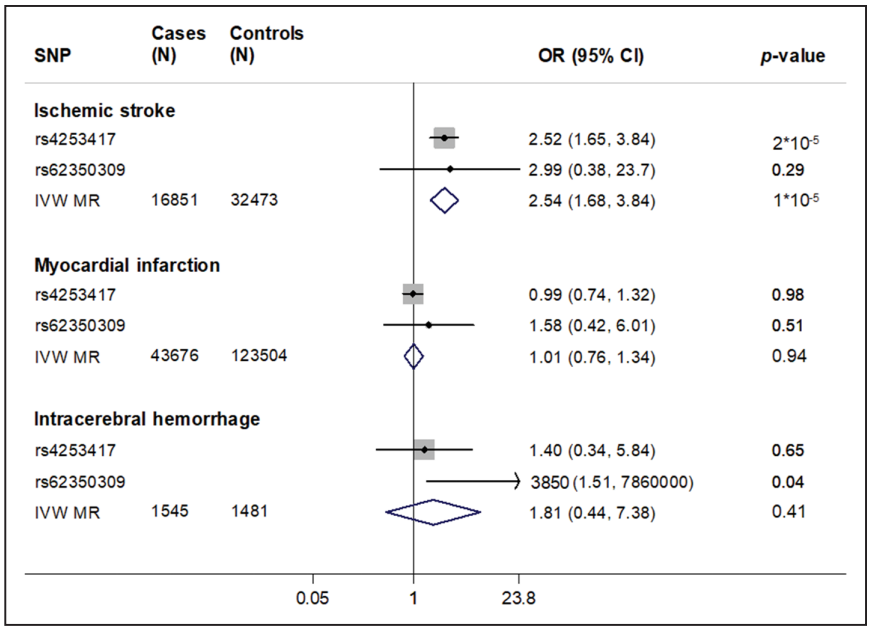

Figure 1. Forest plot of the individual single-nucleotide polymorphism (SNP) and pooled Mendelian randomization (MR) estimates for the causal effect of FXI (factor XI) levels on risk of ischemic stroke, myocardial infarction, and intracerebral hemorrhage in the main analysis. IVW indicates inverse variance; and OR, odds ratio.

of studies used to obtain genetic association estimates for IS, IS subtypes, MI, and ICH are provided in Methods in the online-only Data Supplement. All studies had obtained ethical approval.

Individual MR estimates for each SNP were calculated using the ratio method, with SEs derived with the Delta method.[5] Overall MR estimates were obtained by pooling the MR results of individual SNPs using fixed-effects inverse-variance (IVW) meta-analysis.[5] As a sensitivity analysis, we also performed an unweighted allele score that pooled the SNP-outcome estimates for the main FXI instruments using fixed-effects IVW meta-analysis. Such an approach does not consider the magnitude of effect for each SNP on FXI levels.

As a further sensitivity analysis using more SNPs, we also repeated the IVW MR using relaxed criteria for instrument selection. For this, we selected SNPs across the genome associated with natural logarithm-transformed FXI levels at $P<5\times10^{-6}$ and in low linkage disequilibrium with $r^2<0.01$. Using these instruments, we also performed the MR-Egger sensitivity analysis, which is more robust to the inclusion of pleiotropic instruments and offers a test for the presence of directional pleiotropy.[6] A threshold of $P<0.007$ was used to determine statistical significance for all analyses, after applying a Bonferroni multiple testing correction for the 7 outcomes.

Results

The rs4253417 and rs62350309 SNPs were identified as instruments for FXI levels in the main analyses (Table I in the online-only Data Supplement). Individual MR estimates for each SNP and the overall IVW MR estimates are given in Figure 1 for IS, MI, and ICH and in Figure 2 for the IS subtypes. The 95% CIs overlapped for the 2 SNPs throughout.

The IVW MR analysis supported a causal effect of higher, genetically determined FXI levels on risk of any IS (odds ratio

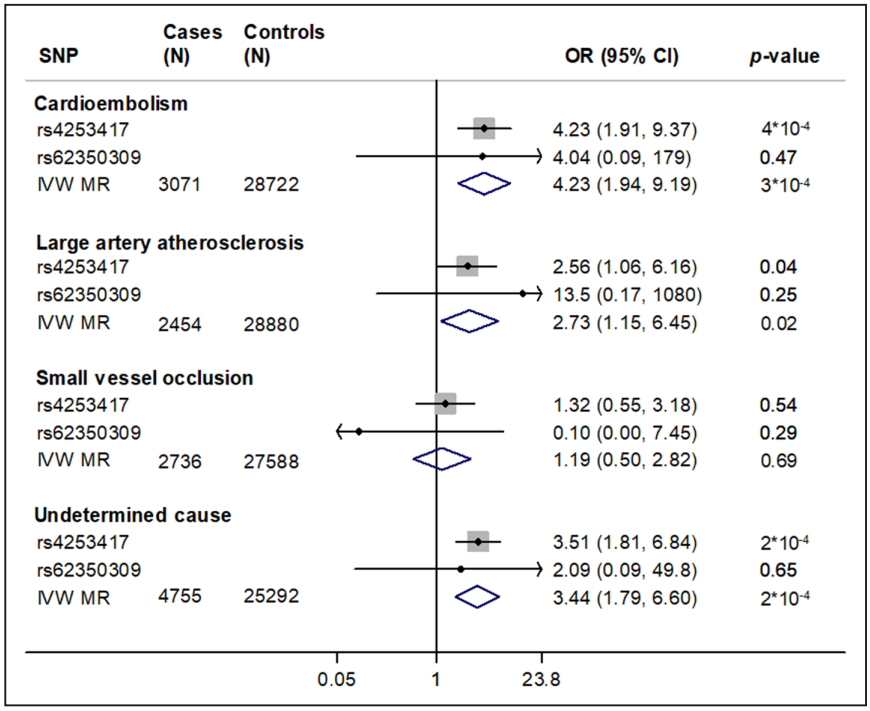

Figure 2. Forest plot of the individual single-nucleotide polymorphism (SNP) and pooled Mendelian randomization (MR) estimates for the causal effect of FXI (factor XI) levels on risk of ischemic stroke etiological subtypes in the main analysis. IVW indicates inverse variance; and OR, odds ratio.

[OR] per 1-unit increase in natural logarithm-transformed FXI levels, 2.54; 95% CI, 1.68–3.84; $P=1\times10^{-5}$) but not MI (OR, 1.01; 95% CI, 0.76–1.34; $P=0.94$) or ICH (OR, 1.81; 95% CI, 0.44–7.38; $P=0.41$; Figure 1). Examining IS subtypes, the results supported a causal effect of higher, genetically determined FXI levels on risk of cardioembolism (CE) (OR, 4.23; 95% CI, 1.94–9.19; $P=3\times10^{-4}$) and IS of undetermined cause (OR, 3.44; 95% CI, 1.79–6.60; $P=2\times10^{-4}$) but not risk of large artery atherosclerosis (OR, 2.73; 95% CI, 1.15–6.45; $P=0.02$) or small artery occlusion (OR, 1.19; 95% CI, 0.50–2.82; $P=0.69$; Figure 2). Results of the unweighted allele score supported the main IVW MR findings, with concordant directions of effect and IS, $P=4\times10^{-4}$; MI, $P=0.73$; ICH, $P=0.69$; CE, $P=0.003$; large artery atherosclerosis, $P=0.14$; small artery occlusion, $P=0.32$; and IS of undetermined cause, $P=0.001$.

Using the more relaxed criteria for instrument selection, we identified 26 SNPs as instruments, with results for only 15 of these available for ICH (Table II in the online-only Data Supplement). IVW MR and MR-Egger using these SNPs supported the findings of the main analysis for any IS and CE (Table III in the online-only Data Supplement). However, statistical significance ($P<0.007$) was not achieved for any of the other outcomes considered (Table III in the online-only Data Supplement) and in particular, IS of undetermined cause (IVW MR: OR, 1.56; 95% CI, 1.11–2.19; $P=0.01$; MR-Egger: OR, 1.61; 95% CI, 0.89–2.92; $P=0.12$). There was no MR-Egger evidence for the presence of directional pleiotropy in any of the analyses (IS, $P=0.75$; MI, $P=0.24$; ICH, $P=0.31$; CE, $P=0.07$; large artery atherosclerosis, $P=0.86$; small artery occlusion, $P=0.10$; and IS of undetermined cause, $P=0.87$).

Discussion

Our findings are consistent with the observational association of individuals with FXI deficiency having a lower risk of IS, but not MI,[1] and also highlight FXI as a possible target to prevent IS. It is interesting to note the MR association of genetically determined FXI levels with IS but not MI, despite existing novel oral anticoagulants proving efficacious for preventing MI. The discrepancy may relate to differences in the action of FXI at various anatomic sites and vascular beds.[1] In terms of the adverse effects associated with FXI level lowering, reduction using a second-generation antisense oligonucleotide to prevent postoperative venous thromboembolism was not associated with excessive bleeding as compared with standard care[2] and similarly, we did not find evidence of a causal effect of genetically determined FXI levels on risk of ICH.

The major strength of our MR study is that it has allowed investigation of the causal effect of genetically determined FXI levels on risk of IS, IS etiological subtypes, MI, and ICH. The main IVW MR analysis maximizes statistical power but is prone to bias from the inclusion of pleiotropic instruments. In contrast, although the use of more relaxed instrument selection criteria in sensitivity analyses possibly introduced bias through introduction of weak or invalid instruments, it allowed for MR-Egger sensitivity analysis (Table III in the online-only Data Supplement), which is more robust to inclusion of pleiotropic instruments under the assumption that instrument strength is independent of any pleiotropic effect.[6] In terms of weaknesses of our study, the negative findings may be related to lack of sufficient statistical power to detect an effect. In addition, we did not investigate bleeding phenotypes other than ICH. Furthermore, our MR analysis assumes a linear effect of natural logarithm-transformed FXI levels and reflects the lifetime cumulative consequence of higher, genetically determined FXI levels. Although the unweighted allele score, which does not consider the magnitude of effect that the instruments had on FXI levels, supported the main analysis, MR results should not be extrapolated to estimate the quantitative effect of clinical intervention. Indeed, this MR study does not provide data on the effect of clinically altering FXI levels.

Summary

Our MR study supports FXI as a possible target for reducing the risk of the CE subtype of IS. Importantly, it suggests a differential effect of genetically determined FXI on the risk of different IS subtypes, which may help future clinical studies target appropriate populations.

Sources of Funding

Dr Gill led this study as part of a Wellcome 4i Clinical PhD Programme at Imperial College London. Dr Sabater-Lleal is supported by a Miguel Servet contract (ISCIII CP17/00142) from the Spanish Ministry of Health and acknowledges funding from the Swedish Heart and Lung Foundation (No. 20160290).

Disclosures

Dr Veltkamp is an investigator of the Imperial Biomedical Research Centre and has received honoraria and research support from Bayer, Bristol-Myers Squibb, Biogen, Boehringer Ingelheim, Daiichi Sankyo, Medtronic, Morphosys, and Pfizer. The other authors report no conflicts.

References

1. Salomon O, Steinberg DM, Koren-Morag N, Tanne D, Seligsohn U. Reduced incidence of ischemic stroke in patients with severe factor XI deficiency. *Blood*. 2008;111:4113–4117. doi: 10.1182/blood-2007-10-120139
2. Büller HR, Bethune C, Bhanot S, Gailani D, Monia BP, Raskob GE, et al; FXI-ASO TKA Investigators. Factor XI antisense oligonucleotide for prevention of venous thrombosis. *N Engl J Med*. 2015;372:232–240. doi: 10.1056/NEJMoa1405760
3. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol*. 2003;32:1–22.
4. Sennblad B, Basu S, Mazur J, Suchon P, Martinez-Perez A, van Hylckama Vlieg A, et al. Genome-wide association study with additional genetic and post-transcriptional analyses reveals novel regulators of plasma factor XI levels. *Hum Mol Genet*. 2017;26:637–649. doi: 10.1093/hmg/ddw401
5. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol*. 2013;37:658–665. doi: 10.1002/gepi.21758
6. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol*. 2015;44:512–525. doi: 10.1093/ije/dyv080