

Spring 5-2019

A MENDELIAN RANDOMIZATION STUDY OF CIRCULATING GAMMA PRIME FIBRINOGEN AND TOTAL FIBRINOGEN LEVELS ON VENOUS THROMBOEMBOLISM AND ISCHEMIC STROKE SUBTYPES

JILLIAN MANERS
UTHealth School of Public Health

Follow this and additional works at: https://digitalcommons.library.tmc.edu/uthsph_dissertsopen



Part of the [Community Psychology Commons](#), [Health Psychology Commons](#), and the [Public Health Commons](#)

Recommended Citation

MANERS, JILLIAN, "A MENDELIAN RANDOMIZATION STUDY OF CIRCULATING GAMMA PRIME FIBRINOGEN AND TOTAL FIBRINOGEN LEVELS ON VENOUS THROMBOEMBOLISM AND ISCHEMIC STROKE SUBTYPES" (2019). *UT School of Public Health Dissertations (Open Access)*. 70.
https://digitalcommons.library.tmc.edu/uthsph_dissertsopen/70

This is brought to you for free and open access by the School of Public Health at DigitalCommons@TMC. It has been accepted for inclusion in UT School of Public Health Dissertations (Open Access) by an authorized administrator of DigitalCommons@TMC. For more information, please contact digitalcommons@library.tmc.edu.

A MENDELIAN RANDOMIZATION STUDY OF CIRCULATING GAMMA PRIME
FIBRINOGEN AND TOTAL FIBRINOGEN LEVELS ON VENOUS
THROMBOEMBOLISM AND ISCHEMIC STROKE SUBTYPES

by

JILLIAN MANERS, BS

APPROVED:

CATHERINE TROISI, PHD

PAUL DE VRIES, PHD

Copyright
by
Jillian Maners, BS, MPH
2019

DEDICATION

To David and Bernice Maners

A MENDELIAN RANDOMIZATION STUDY OF CIRCULATING GAMMA PRIME
FIBRINOGEN AND TOTAL FIBRINOGEN LEVELS ON VENOUS
THROMBOEMBOLISM AND ISCHEMIC STROKE SUBTYPES

by

JILLIAN MANERS
BS, Texas A&M University, 2015

Presented to the Faculty of The University of Texas

School of Public Health

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF PUBLIC HEALTH

THE UNIVERSITY OF TEXAS
SCHOOL OF PUBLIC HEALTH
Houston, Texas
May 2019

ACKNOWLEDGEMENTS

Thank you to my grandparents, whose generosity has enabled me to pursue my MPH and other passions; to my parents, who have consistently supported me wherever my ambitions have taken me; and to Dr. Troisi for encouraging me to embark on this research project.

Thank you, Dr. de Vries, for continually pushing me beyond what I thought I was capable of during this research experience. I will be a better epidemiologist because of your patience and persistence.

Lastly, I would like to thank the coauthors that have helped make this work possible. This includes Paul de Vries, Dipender Gill, Nathan Pankratz, Mike Laffan, Alisa Wolberg, Moniek de Maat, Weihong Tang, Cavin Ward-Caviness, Nicholas Smith, Alanna Morrison, and Abbas Dehghan. I would also like to thank the CHARGE Hemostasis Working Group, MEGASTROKE consortium of the International Stroke Genetics Consortium, and the INVENT consortium for their sharing of summary statistics pertaining to various genome-wide association studies.

ABSTRACT: A MENDELIAN RANDOMIZATION STUDY OF CIRCULATING
GAMMA PRIME FIBRINOGEN AND TOTAL FIBRINOGEN LEVELS ON VENOUS
THROMBOEMBOLISM AND ISCHEMIC STROKE SUBTYPES

Jillian Maners, MPH, BS
The University of Texas
School of Public Health, 2019

Thesis Chair: Paul de Vries, PhD

Introduction: Fibrinogen is a key component of the coagulation cascade, and variation in its circulating levels may contribute to thrombotic diseases, such as venous thromboembolism (VTE) and ischemic stroke. Gamma prime (γ') fibrinogen is an isoform of fibrinogen and has anticoagulant properties, including inhibiting thrombin and slowing platelet aggregation, that may affect clot formation.

Methods: Two-sample Mendelian randomization (MR) was applied to estimate the causal effect of total circulating fibrinogen and its isoform, γ' fibrinogen, on risk of VTE and ischemic stroke subtypes using summary statistics from published genome-wide association studies of fibrinogen, VTE, and ischemic stroke, and an unpublished study of γ' fibrinogen. Genetic instruments for γ' fibrinogen and total fibrinogen were selected by pruning genome-wide significant variants to linkage disequilibrium $r^2 < 0.1$. The inverse variance weighted MR approach was used to estimate effects in the main analysis, with additional approaches that are more robust to the inclusion of pleiotropic variants applied in sensitivity analyses, including MR-Egger, weighted median MR, and weighted mode MR.

Results: The main inverse variance weighted MR estimates (**Figure 1**) based on 16 genetic instruments for γ' fibrinogen and 75 genetic instruments for total fibrinogen (**Figure 2**) indicated a protective effect of both γ' fibrinogen and total fibrinogen on VTE risk. Higher γ' fibrinogen levels decreased the risk of cardioembolic and large artery stroke. Higher total fibrinogen levels decreased the risk of cardioembolic stroke but increased the risk of large artery and small vessel stroke. Effect estimates were consistent across sensitivity analyses, indicating that the results are unlikely to be attributable to the inclusion of pleiotropic variants.

Conclusion: Our results are consistent with effects of genetically determined γ' fibrinogen on VTE and ischemic stroke. The strong inverse association found between γ' fibrinogen and VTE and ischemic stroke subtypes suggests the need to evaluate γ' fibrinogen's causal effect on other cardiovascular outcomes. Further research is needed to explain the protective effects seen in total fibrinogen on VTE and on cardioembolic stroke.

TABLE OF CONTENTS

List of Tables	i
List of Figures	ii
List of Appendices	iii
Background	1
Literature Review.....	1
Hypothesis, Research Question, Specific Aims or Objectives	5
Public Health Significance.....	6
Journal Article.....	7
Title of Journal Article: A mendelian randomization study of circulating gamma prime fibrinogen and total fibrinogen levels on venous thromboembolism and ischemic stroke subtypes	7
Name of Journal Proposed for Article Submission: Blood.....	7
Conclusion	21
Appendices.....	25
References.....	40

LIST OF TABLES

Table 1: Summary of genome-wide association studies used in the summary statistics based two sample Mendelian randomization analyses.	10
---	----

LIST OF FIGURES

- Figure 1: Forest plot of the association of genetically determined γ' fibrinogen levels with venous thromboembolism, ischemic stroke and its subtypes, using the inverse variance weighted method.....14
- Figure 2: Forest plot of the association of genetically determined total fibrinogen levels with venous thromboembolism, ischemic stroke and its subtypes, using the inverse variance weighted method15

LIST OF APPENDICES

Appendix A: Manhattan Plot for the genome-wide association study of γ' fibrinogen levels	25
Appendix B: Causal effect estimate produced by individual genetic instruments for γ' fibrinogen levels.....	26
Appendix C: Sensitivity analyses for Mendelian randomization of γ' fibrinogen levels	259
Appendix D: Causal effect estimate produced by individual genetic instruments for total fibrinogen levels	325
Appendix E: Sensitivity analyses for Mendelian randomization of total fibrinogen levels	37

BACKGROUND

Genome-wide association studies (GWAS) for venous thromboembolism (VTE) and ischemic stroke have identified significant associations with genetic variants at the *FGA*, *FGB*, and *FGG* genes (Germain et al., 2015; Malik et al., 2018). These genes encode the subunits of a protein called fibrinogen and highlight fibrinogen as a protein that may have a role in the pathophysiology of these cardiovascular outcomes.

Literature Review

Pathophysiology of VTE and ischemic stroke subtypes

VTE usually results from a fibrin-rich blood clot formed in the veins of the leg and is identified as either deep vein thrombosis or as pulmonary embolism (Wolberg, Monroe, Roberts, & Hoffman, 2003). Pulmonary embolism is a complication of deep vein thrombosis where the clot breaks apart and travels up into the smaller blood vessels of the lung, causing a blockage (Patel et al., 2017). Although deep vein thrombosis often results from the stasis of blood in the deep veins, some acute incidences result from hypercoagulability (Wolberg et al., 2003). Ischemic stroke, caused by blood vessel blockage in the brain, has several different subtypes, including cardioembolic stroke, large artery stroke, and small vessel stroke resulting from small vessel disease (Malik et al., 2018). Cardioembolic stroke occurs when a clot in an artery of the heart forms and travels to obstruct a vessel in the brain (Adams et al., 1993). Cardioembolic strokes can be caused by cardiac abnormalities, arrhythmias, or improperly functioning heart valves (O'Carroll & Barrett, 2017). Large artery stroke usually results from a clot that form in an atherosclerotic artery: when an

atherosclerotic plaque ruptures, this triggers the formation of a blood clot that then travels to the brain and obstructs one of the large arteries (Banerjee & Turan, 2017). Small vessel stroke is caused by occlusion of the small vessels in the brain (Traylor et al., 2015). Although the pathophysiology is not completely understood, small vessel strokes resulting from small vessel disease is associated with hypertension and seems to be highly heritable (Khan, Porteous, Hassan, & Markus, 2007; Traylor et al., 2015).

Properties and function of fibrinogen

Fibrinogen is a precursor of the fibrin mesh that forms blood clots; it also promotes platelet aggregation and acts as a regulator in inflammation (Davalos & Akassoglou, 2012; Mosesson, 2005). The fibrinogen molecule is encoded by *FGA*, *FGB*, and *FGG* genes that respectively form two of each of the following subunits: $A\alpha$, $B\beta$, and γ (Omarova et al., 2013). The standard version of the γ chain is called γA , and typical fibrinogen includes two γA chains. Alternative splicing of the *FGG* transcript, however, results in a γ' polypeptide chain that forms $\gamma A/\gamma'$ fibrinogen and γ'/γ' fibrinogen, which are collectively known as gamma prime (γ') fibrinogen and compose up to 15% of total circulating fibrinogen levels (Omarova et al., 2013). The γ' chain has a different carboxyl-terminal region than that of the typical γA chain, and this appears to confer it with several anticoagulant properties. This region in the γ' chain binds thrombin with high-affinity and inhibits factor XIII that acts to stabilize a clot (Chung & Davie, 1984; Mosesson, 2003). Thrombin binding to the γ' chain suppresses thrombin generation, resulting in decreased rates of fibrin formation and of

platelet aggregation (Lancellotti et al., 2008; Mosesson, 2005; Sidelmann, Gram, Jespersen, & Kluft, 2000).

Indeed, even before the first GWAS was published, a haplotype in the *FGG* gene (FGG-H2) was found to be associated with lower levels of γ' fibrinogen and a higher risk of VTE (Uitte de Willige et al., 2005). This result was later recapitulated by GWAS of VTE (Germain et al., 2015). More recently, a large GWAS of ischemic stroke has identified this same signal to also be associated with ischemic stroke, with the γ' fibrinogen lowering allele being associated with a higher risk of ischemic stroke (Malik et al., 2018). This provides a rationale for suspecting that the apparent involvement of fibrinogen in the pathophysiology of VTE and ischemic stroke may be explained by the anticoagulant properties of γ' fibrinogen.

Epidemiological studies

A case-control study with γ' fibrinogen suggests an inverse association with deep vein thrombosis (Uitte de Willige et al., 2005). However, large prospective cohort studies, did not find an association between γ' fibrinogen and VTE or stroke (Appiah, Schreiner, MacLehose, & Folsom, 2015; Appiah, Heckbert, Cushman, Psaty, & Folsom, 2016; Folsom et al., 2016).

While a few case-control studies showed a correlation of total circulating fibrinogen with VTE risk, (Koster et al., 1994; Yamashita et al., 2014) other studies were unable to find an association (Hunt et al., 2018; Payne, Miller, Hooper, Lally, & Austin, 2014; Tsai et al., 2002). With regards to ischemic stroke, elevated total fibrinogen levels have been associated

with an increased risk of ischemic stroke in a few prospective cohort studies (Siegerink, Rosendaal, & Algra, 2009) and case control studies (Kim et al., 2013; Zhang et al., 2013).

Overcoming Limitations Inherent in Observational Studies

Contradictions in the literature lead to more questions about how γ' fibrinogen and total fibrinogen levels influence the risk of VTE and ischemic stroke. However, associations of γ' fibrinogen and total fibrinogen with VTE and ischemic stroke detected in observational studies may have been influenced by confounding and reverse causation. Mendelian randomization (MR) can be used to overcome these limitations, by using genetic variants associated with fibrinogen levels as instrumental variables to assess the causal effect of γ' fibrinogen and total fibrinogen levels on VTE and ischemic stroke. In an MR, genetic variants that measure a particular exposure are identified in order to make causal inferences regarding the effect of the exposure on the outcome (Davey Smith & Hemani, 2014). The genetic variants can be identified in a GWAS.

Because of the independent assortment of alleles at conception, genetic variants are independent of environmental or behavioral factors, such that they do not act as confounders. Reverse causation is another limitation that is present in observational studies that MR seeks to overcome. Because genetics precedes incidence of disease, and disease cannot change genetics, interpretation of results are not limited by the possibility of reverse causation. Nevertheless, for an instrumental variable to be valid for MR, several assumptions must be made. First of all, the genetic instrument must be associated with the exposure. Secondly, the genetic instrument can only affect the outcome through the exposure being measured. The

instrument cannot directly impact disease or contribute to the disease in question through another pathway, nor can it be associated with another cause of the disease (Haycock et al., 2016). A genetic variant that is being used as an instrumental variable can violate this assumption by having pleiotropic effects on other phenotypes other than the main exposure of interest.

The observations discussed above about γ' fibrinogen lowering variants in the FGG-H2 haplotype increasing the risk of VTE and ischemic stroke already constitute a kind of pseudo-MR, but the application of formal MR methodology has many advantages, including that it allows for an estimation of the causal effect size.

Hypothesis, Research Question, Specific Aims or Objectives

This project aims to whether or not there is a significant causal association of γ' fibrinogen and total fibrinogen levels with VTE and ischemic stroke. Due to the anticoagulant nature of γ' fibrinogen, we hypothesized that elevated levels of genetically determined γ' fibrinogen levels would decrease risk of VTE and ischemic stroke. Related to total fibrinogen, we hypothesized that elevated levels of genetically determined total fibrinogen would increase the risk of VTE and ischemic stroke based on published observational studies. To test our hypotheses, we performed two-sample MR leveraging the results of large-scale GWAS, to explore the causal effects γ' fibrinogen and total fibrinogen on VTE and ischemic stroke. If a causal association is discovered, this information could be used to determine potential lifestyle interventions or drug targets used to prevent or treat VTE and ischemic stroke.

Public Health Significance

The importance of γ' fibrinogen is underappreciated, as evidenced by the relative lack of observational studies evaluating its association with cardiovascular outcomes. The anticoagulant effects of γ' fibrinogen signify the need to evaluate how levels of γ' fibrinogen impact cardiovascular outcomes, such as VTE and ischemic stroke. Knowing if, or how, genetically determined γ' fibrinogen levels influence risk of VTE or ischemic stroke could pave the way for new drug targets to be used in the prevention and/or treatment of these outcomes.

Total fibrinogen has been implicated in observational studies regarding cardiovascular outcomes. Understanding its association with cardiovascular outcomes, like VTE or stroke is important, and just like with γ' fibrinogen levels, the discovery of a causal effect of total fibrinogen levels on these outcomes could provide the foundation for the creation of new therapeutic interventions.

JOURNAL ARTICLE

Title of Journal Article: A Mendelian randomization study of circulating gamma prime fibrinogen and total fibrinogen levels on venous thromboembolism and ischemic stroke subtypes

Name of Journal Proposed for Article Submission: Blood

Introduction

Fibrinogen is a key protein in coagulation, as it serves as the precursor of fibrin and promotes platelet aggregation (Sidelmann et al., 2000). Fibrinogen is also an inflammatory mediator that increases in concentration upon infection, injury, or other trauma leading to inflammation (Davalos & Akassoglou, 2012).

Gamma prime (γ') fibrinogen is a protein resulting from alternative splicing at the fibrinogen locus and makes up about 8-15% of total plasma fibrinogen (Farrell, 2012). γ' fibrinogen has several anticoagulant properties that it does not share with other forms of fibrinogen (Chung & Davie, 1984; Mosesson, 2003).

Given fibrinogen's role in coagulation, γ' fibrinogen and total fibrinogen levels may affect the risk of arterial and venous thrombosis. Indeed, some observational studies have found higher total fibrinogen levels to be associated with an increased risk of venous thromboembolism (VTE) (Koster et al., 1994; Yamashita et al., 2014) and stroke (Chuang, Bai, Chen, Lien, & Pan, 2009; Kim et al., 2013; Siegerink et al., 2009; Zhang et al., 2013), although these associations are not consistent across all studies (Hunt et al., 2018; Tsai et al., 2002). A case-control study suggests an inverse association with γ' fibrinogen and venous

thrombosis (Uitte de Willige et al., 2005). Large prospective cohort studies, however, did not find an association between γ' fibrinogen and VTE or stroke (Appiah et al., 2015; Appiah et al., 2016; Folsom et al., 2016). In genome-wide association studies (GWAS) of VTE and ischemic stroke, the locus encoding the fibrinogen subunit genes was found to be genome-wide significant (Germain et al., 2015; Malik et al., 2018), and the variant that was associated with a higher risk of these outcomes was associated with lower levels of γ' fibrinogen (Uitte de Willige et al., 2005).

With observational studies the association of fibrinogen and γ' fibrinogen with VTE and stroke may have been influenced by confounding and reverse causation. To combat this, we can use Mendelian randomization (MR) to explore the causal effects of γ' fibrinogen and total fibrinogen levels on VTE and ischemic stroke by leveraging the results of large-scale GWAS). We used two-sample MR to leverage genome-wide significant associations discovered in GWAS for the determination of causal effects of γ' and total fibrinogen levels on VTE and ischemic stroke subtypes.

Methods

Included published GWAS

The MR analyses used summary statistics from several GWAS, which are described in **Table 1**. All of these GWAS were restricted to European-ancestry participants and were based on genotypes imputed using the 1000 Genomes Project phase 1 version 3 reference panel. We used a GWAS of circulating fibrinogen concentration by the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE). This study included 120,246 participants and

identified 41 genome-wide significant loci that explained 3% of variance in circulating plasma fibrinogen (de Vries et al., 2016). Summary statistics were then used to determine the effect of genetic variants on fibrinogen levels.

To assess the effect of genetic variants on VTE, we used summary statistics from the largest published GWAS on VTE, by the International Network against VENous Thrombosis (INVENT) consortium, based on 7,507 VTE cases and 52,632 controls (Germain et al., 2015).

To assess the effect of genetic variants on ischemic stroke and its subtypes (cardioembolic strokes, large artery stroke, and small vessel stroke) a GWAS by the MEGASTROKE consortium based on 67,162 cases and 454,450 controls was used (Malik et al., 2018) (**Table 1**). The GWAS for stroke and VTE were restricted to European-ancestry individuals and based on genotype data imputed to the European samples from phase 3 of the 1000 Genomes Project.

GWAS of γ' fibrinogen

Summary statistics from GWAS based on the 1000 Genomes Project reference panel were not available for γ' fibrinogen. Therefore, we performed a GWAS of circulating γ' fibrinogen concentration in European-ancestry participants from the Atherosclerosis Risk in Communities (ARIC) study. γ' fibrinogen (mg/dL) was measured from fasting citrated plasma collected at ARIC visit 3 using ELISA, as described previously (Appiah et al., 2015). γ' fibrinogen levels were natural log transformed prior to analyses. Outliers with γ' fibrinogen levels ± 3 SD from the mean were excluded, resulting in a final sample size of 9,225

participants. Genotypes were assessed using the Affymetrix (Santa Clara, CA, USA) Genome-wide Human SNP Array 6.0 assay, and additional genotypes were imputed using IMPUTE2 (Howie, Marchini, & Stephens, 2011; Howie, Fuchsberger, Stephens, Marchini, & Abecasis, 2012) based on the 1000 Genomes Project phase 1 version 3 reference panel (1000 Genomes Project Consortium et al., 2012).

Genome-wide analyses were then performed using FAST version 1.8 (Chanda, Huang, Arking, & Bader, 2013), adjusted for age, sex, and ancestry-informative principal components 1 and 2. A total of 9,335,343 variants with minor allele frequency $> 1\%$ and imputation quality $r^2 > 0.3$ were considered in the analysis.

Table 1: Summary of genome-wide association studies used in the summary statistics based two sample Mendelian randomization analyses.

Phenotype	Sample Size	Consortium	Reference
γ' fibrinogen levels	9,225	ARIC study	Unpublished
Total fibrinogen levels	120,246	CHARGE consortium	de Vries et al.
Venous thromboembolism	7,507 cases / 52,632 controls	INVENT consortium	Germain et al.
Cardioembolic stroke	7,193 cases / 322,150 controls	MEGASTROKE	Malik et al.
Large artery stroke	4,373 cases / 204,991 controls	MEGASTROKE	Malik et al.
Small vessel stroke	5,386 cases / 254,558 controls	MEGASTROKE	Malik et al.
Any ischemic stroke	34,217 cases / 434,418 controls	MEGASTROKE	Malik et al.

Genetic instruments

We separately applied the following steps to the summary statistics for circulating γ' fibrinogen and fibrinogen levels. In order to obtain sets of independent genetic instruments

for each of these exposures, we selected all genome-wide significant variants ($p < 5 \times 10^{-8}$) and pruned these variants according to their pairwise linkage disequilibrium (LD) by removing the variant with the highest p-value from each pair of variants with a LD $r^2 > 0.1$.

Mendelian randomization - primary analysis

The TwoSampleMR package implemented in R was used to calculate estimates from genetic instruments of the causal effect of the corresponding exposure (γ' fibrinogen or total fibrinogen levels) on each of the outcomes VTE, , cardioembolic stroke, large artery stroke, small vessel stroke, and any ischemic stroke). Evidence from each of the genetic instruments was then combined using inverse-variance weighted (IVW) meta-analysis to produce a single estimate of the causal effect of each exposure on each outcome (Burgess, Butterworth, & Thompson, 2013).

Mendelian randomization - sensitivity analyses

Several sensitivity analyses were performed, including MR-Egger, weighted median estimator, and weighted mode estimator, each being robust to certain kinds of pleiotropy. If there is horizontal pleiotropy within the instrumental variable, a bias causal estimate can result. MR-Egger is used assesses whether the effect estimate is influenced by directional pleiotropy. It can be used even when all of the instruments are pleiotropic (Bowden, Davey Smith, & Burgess, 2015). Weighted median estimator is a more powerful and precise approach than MR-Egger, but it only gives a consistent causal effect estimate when no more than half of the instruments are invalid (Bowden, Davey Smith, Haycock, & Burgess, 2016).

The weighted mode estimator gives consistent causal effect estimates in the presence of horizontal pleiotropy, but only when weights associated with the valid instruments are the largest (Hartwig, Davey Smith, & Bowden, 2017).

We used the global test implemented in the MR-PRESSO R package to detect heterogeneity amongst the genetic instruments. When the global test was significant (p-value <0.05) we removed statistically significant outliers detected by MR-PRESSO (p-value <0.05) and repeated the IVW analysis. As an additional sensitivity analysis, the Mendelian Randomization R package was used to perform IVW MR analyses corrected for LD between genetic instruments (Burgess et al., 2013). Although genetic instruments were selected to be independent ($r^2 < 0.1$), this method was used to determine the effects of residual LD on our primary IVW analyses. As a final sensitivity analysis, we performed an MR using only variants at the fibrinogen gene cluster. Given that the genes near this variant encode the subunits of the fibrinogen protein, they may be more likely to be free of pleiotropic effects that do not go through fibrinogen levels.

Results

GWAS of γ' fibrinogen

In our GWAS of γ' fibrinogen, the only locus that was associated at genome-wide significance ($p < 5 \times 10^{-8}$) was the fibrinogen gene cluster itself, as shown in the Manhattan plot in **Appendix A**. A total of 509 variants at this locus were associated with γ' fibrinogen. The most significant variant at this locus was rs7654093 with a minor allele frequency of

24%. This variant was associated with a 0.2 log(mg/dL) decrease in γ' fibrinogen levels per copy of the minor allele ($p = 3.1 \times 10^{-418}$).

Selection of genetic instruments

After pruning the 5,076 genome-wide significant variants for total fibrinogen levels according to an LD r^2 threshold of 0.1, 75 independent genetic variants remained and were used as genetic instruments for total fibrinogen levels. 16 of these 75 variants were located at the fibrinogen gene cluster. After pruning the 509 genome-wide significant variants for γ' fibrinogen levels, all of which were located at the fibrinogen gene cluster, 16 independent genetic variants remained and were used as genetic instruments for γ' fibrinogen levels.

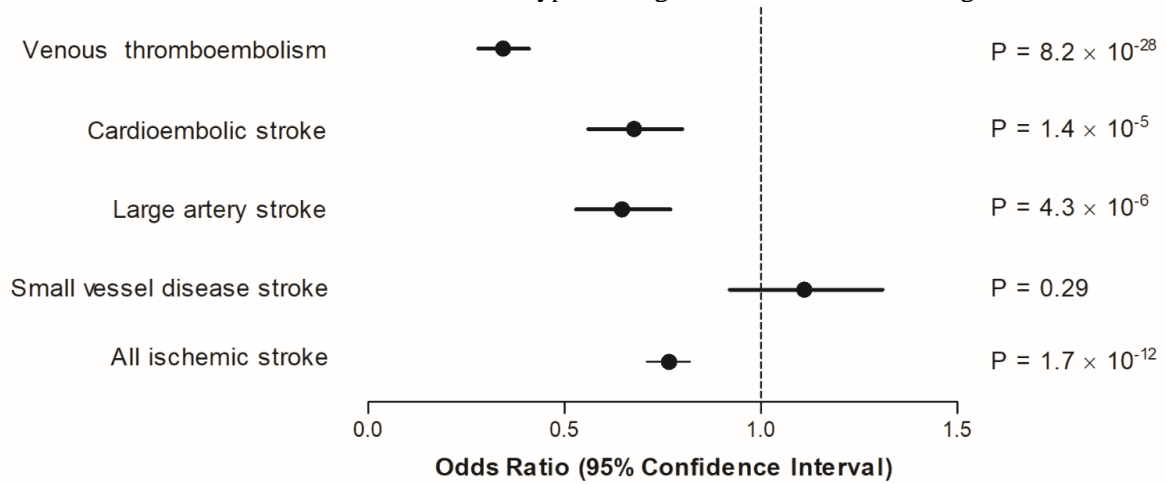
MR of γ' fibrinogen

Forest plots that show the causal effect estimate produced using each individual genetic instrument for γ' fibrinogen levels are shown in **Appendix B**. Effect estimates of genetically determined circulating γ' fibrinogen levels on VTE and stroke subtypes, generated using the IVW approach, are shown in **Figure 1**. Genetically determined γ' fibrinogen levels were inversely associated with the risk of VTE ($OR_{IVW} = 0.34$; $p_{IVW} = 8.2 \times 10^{-28}$), and this association remained consistent across all sensitivity analyses (**Appendix C Figure 1**).

Genetically determined γ' fibrinogen levels were also inversely associated with the risk of cardioembolic stroke ($OR_{IVW} = 0.67$; $p_{IVW} = 1.4 \times 10^{-5}$), large artery stroke ($OR_{IVW} = 0.64$ with a $p_{IVW} = 4.3 \times 10^{-6}$), and any ischemic stroke ($OR_{IVW} = 0.77$; $p_{IVW} = 1.7 \times 10^{-12}$).

These associations had consistent effect size and directions across sensitivity analyses (**Appendix C**). As shown in **Appendix C Figure 4**, genetically determined circulating γ' fibrinogen levels were not associated with small vessel disease ($OR_{IVW} = 1.10$ with a $p_{IVW} = 0.29$)

Figure 1: Forest plot of the association of genetically determined γ' fibrinogen levels with venous thromboembolism, ischemic stroke and its subtypes, using the inverse variance weighted method



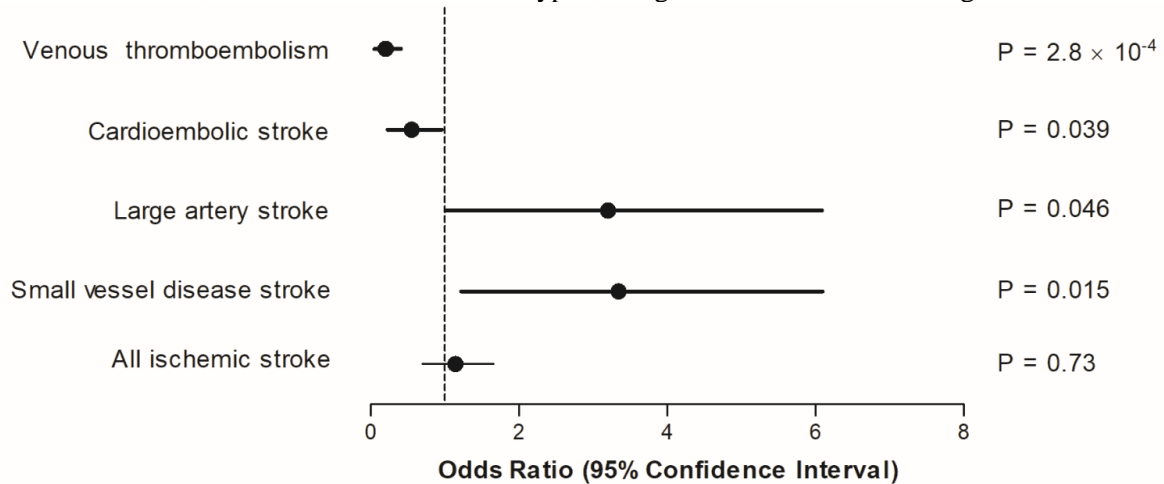
MR of total fibrinogen

Forest plots that show the causal effect estimate produced using each individual genetic instrument for total fibrinogen levels are shown in **Appendix D**. Effect estimates of genetically determined total circulating fibrinogen levels on VTE and stroke subtypes, generated using the IVW approach, are shown in **Figure 2**. Genetically determined total fibrinogen levels were inversely associated with the risk of VTE ($OR_{IVW} = 0.15$, $p_{IVW} = 2.8 \times 10^{-4}$). As seen in **Appendix E Figure 1**, the effect estimates of the association between fibrinogen and VTE were consistent across all sensitivity analyses. Similarly, we found a significant inverse association between genetically determined fibrinogen levels

and cardioembolic stroke ($OR_{IVW} = 0.47$, $p_{IVW} = 0.039$), which was also relatively consistent across sensitivity analyses (**Appendix E Figure 2**).

Genetically determined fibrinogen levels were positively associated with small vessel stroke ($OR_{IVW} = 2.72$, $p_{IVW} = 0.015$) and large artery stroke ($OR_{IVW} = 2.49$, $p_{IVW} = 0.046$). Sensitivity analyses were consistent with a positive effect direction for small vessel stroke (**Appendix E Figure 4**), but did not consistently show a positive effect direction for large artery stroke (**Appendix E Figure 3**). Finally, genetically determined fibrinogen levels were not clearly associated with any ischemic stroke (**Figure 2 and Appendix E Figure 5**).

Figure 2: Forest plot of the association of genetically determined total fibrinogen levels with venous thromboembolism, ischemic stroke and its subtypes, using the inverse variance weighted method



Discussion

In this MR study we examined the effects of genetically determined circulating γ' fibrinogen and total fibrinogen levels and VTE and ischemic stroke subtypes. We found that genetically determined circulating γ' fibrinogen levels were associated with VTE, cardioembolic stroke,

and large artery stroke. Genetically determined total circulating fibrinogen levels were associated with VTE, cardioembolic stroke, and small vessel stroke.

Comparison with observational studies

There are conflicting results in observational studies examining the association of γ' fibrinogen levels with VTE and ischemic stroke have yielded conflicting results. A large longitudinal study was unable to confirm that γ' fibrinogen levels were associated with increased risk of VTE (Folsom et al., 2016). However, we found that high genetically determined γ' fibrinogen levels were associated with lower risk of VTE. With regards to stroke, case-control studies have proposed an association between ischemic stroke and higher levels of γ' fibrinogen (Cheung et al., 2008; van den Herik et al., 2011). However, prospective cohort studies were unable to find an association between plasma γ' fibrinogen and ischemic stroke or any other incident cardiovascular endpoint, despite its apparent role in clot formation (Appiah et al., 2015; Appiah et al., 2016). From these studies it has been suggested that γ' fibrinogen levels may increase in response to inflammation resulting from an incident cardiac event, such as ischemic stroke. In contrast to these results from observation studies, we found that high genetically determined γ' fibrinogen levels were associated with a lower risk of cardioembolic and large artery stroke.

There are conflicting results in observational studies examining the association of total fibrinogen levels with VTE and ischemic stroke. While a few case-control studies showed a positive correlation of plasma fibrinogen with VTE risk, (Koster et al., 1994; Yamashita et al., 2014) other studies were unable to find an association, (Hunt et al., 2018;

Payne et al., 2014; Tsai et al., 2002). In contrast, our MR results consistently suggest that high genetically determined fibrinogen levels are associated with lower risk of VTE. With regards to stroke, elevated fibrinogen levels have been associated with ischemic stroke in a few cohort studies (Chuang et al., 2009; Fibrinogen Studies Collaboration et al., 2005; Siegerink et al., 2009) and case control studies (Kim et al., 2013; Zhang et al., 2013). In our study, genetically determined fibrinogen levels were consistently associated with cardioembolic stroke and small vessel disease. While, for cardioembolic stroke, high genetically determined fibrinogen levels indicate a protective effect, small vessel stroke shows an increased risk associated with high genetically determined fibrinogen levels. High genetically determined total fibrinogen levels were associated with an increased risk of large artery stroke, but the association was not consistent across sensitivity analyses, suggesting that this observation does not reflect a causal effect and was instead driven by the inclusion of pleiotropic genetic instruments.

Interpretation of γ' fibrinogen MR results

γ' fibrinogen has a certain pro-thrombotic property: clots comprised of fibrin derived from γ' fibrinogen have an altered structure, making them more resistant to fibrinolysis (Collet, Nagaswami, Farrell, Montalescot, & Weisel, 2004; Siebenlist et al., 2005). Perhaps more interesting is γ' fibrinogen's anti-thrombotic properties.

γ' fibrinogen has several anticoagulant properties that may explain the observed association of γ' fibrinogen levels with VTE and certain ischemic stroke subtypes. First of all, the use of in vitro assays has suggested that γ' fibrinogen may have an anticoagulant effect by

inhibiting factor V and factor XIII activation (Mosesson, 2003; Omarova et al., 2013). Also, γ' fibrinogen has a high affinity for binding to thrombin, decreasing the amount of circulating thrombin (de Bosch, Mosesson, Ruiz-Saez, Echenagucia, & Rodriguez-Lemoin, 2002; Uitte de Willige et al., 2005). In binding to thrombin, γ' fibrinogen reduces thrombin's enzymatic ability to transform fibrinogen into a fibrin (Lovely, Boshkov, Marzec, Hanson, & Farrell, 2007). Higher levels of γ' fibrinogen would increase the number of thrombin binding sites, thereby decreasing risk of a clot. Because of thrombin's role in activating platelets, γ' fibrinogen's inactivation of thrombin may also interfere with platelet aggregation (Lancellotti et al., 2008). γ' fibrinogen's anticoagulant properties give the expectation that increased levels of γ' fibrinogen would result in decreased risk of thrombotic activity, such as VTE or ischemic stroke.

Interpretation of total fibrinogen MR results

Because of its role in clot formation and platelet aggregation, we would expect to see increased levels of total fibrinogen associated with increased risk of VTE and ischemic stroke (Mosesson, 2005). This would have been consistent with the modest but positive association between genetically observed total fibrinogen levels and coronary heart disease that has been observed in a recent MR (Ward-Caviness et al., 2018). For small vessel stroke this is what we observe. However, for large artery stroke, some sensitivity analyses indicate a positive effect and others indicate a negative effect. Inconsistencies across sensitivity analyses likely indicate the presence of pleiotropic variants in our genetic instrument, so we cannot interpret the effect of total fibrinogen levels on large artery stroke. High circulating

total fibrinogen levels were associated with lowered risk of VTE and cardioembolic stroke in our MR analysis. It is possible that the protective effect we are seeing in our results of total fibrinogen levels on VTE and cardioembolic stroke is due to γ' fibrinogen or other confounding variables. Use of multivariable MR could help determine if total fibrinogen's protective effect on VTE and cardioembolic stroke is being mediated by pleiotropic effects of the instrumental variables (Burgess & Thompson, 2015).

Another reason why we may be seeing a protective effect is that higher levels of total fibrinogen may increase clot stabilization, preventing embolism (Wolberg et al., 2003). Pulmonary embolism, a manifestation of VTE, and cardioembolic stroke involve a clot breaking off and travelling to obstruct a blood vessel.

Strengths & Limitations

MR has several strengths compared to observational epidemiological research, including reduced susceptibility to confounding and reverse causation. In our MR, the effects of the genetic instruments on VTE and ischemic stroke subtypes were obtained using the largest GWAS available of these outcomes (Germain et al., 2015; Malik et al., 2018). Additionally, genetic instruments for fibrinogen came from the largest, most well-powered GWAS of fibrinogen currently available (de Vries et al., 2016). Instead of relying on a single genetic instrument, we combined information across multiple variants and loci, allowing us to further increase our power.

Horizontal pleiotropy and linkage disequilibrium among the genetic instruments can lead to violation of critical assumptions of MR, which can lead to biased effect estimates

(Davey Smith & Hemani, 2014). To minimize the impact of horizontal pleiotropy among the genetic instruments, we used a wide variety of sensitivity analyses, each with their own assumptions about the pleiotropic nature of the instruments. Without knowing the number of pleiotropic SNPs and the extent to which they are violating this assumption, we cannot know which sensitivity analysis is most accurate. However, a broad consensus across sensitivity analyses suggests that the impact of pleiotropy is minimal, and this was the case for the majority of the reported associations. We used multiple variants at the fibrinogen gene cluster as genetic instruments, including uncorrelated instruments according to a threshold of $r^2 = 0.1$. To control for potential bias related to residual LD ($r^2 < 0.1$) among these variants, we performed a separate sensitivity analysis designed to account for LD, and this did not greatly impact our results.

Caution should be exercised when interpreting these results, as effect estimates are given in per log(g/L) change for fibrinogen and per log(mg/dL) change for γ' fibrinogen. A linear effect can therefore, not be assumed. Finally, the GWAS of γ' fibrinogens was conducted in a much smaller sample size than the GWAS of fibrinogen levels. As a result, the only genome-wide significant locus was the fibrinogen gene cluster. However, this was the largest available GWAS of γ' fibrinogen levels available at the time of the analyses. If a larger GWAS of γ' fibrinogen levels becomes available in the future, this would increase the power to detect causal connections between γ' fibrinogen and cardiovascular outcomes.

Conclusion

We found that high genetically determined γ' fibrinogen levels were associated with decreased risk of VTE, cardioembolic stroke, and large artery stroke, and that high genetically determined total fibrinogen was associated with decreased risk of VTE and cardioembolic stroke, but an increased risk of small vessel stroke. The association of γ' fibrinogen levels with VTE and cardioembolic stroke was expected, given γ' fibrinogen's anticoagulant properties, but we did not anticipate the negative association of total fibrinogen levels with VTE and cardioembolic stroke. Further research is required to explain this apparent protective effect of total fibrinogen levels on these outcomes.

CONCLUSION

Our aim was to assess whether or not there is a causal association of γ' fibrinogen and total fibrinogen levels on VTE and ischemic stroke. We hypothesized that increased levels of genetically determined γ' fibrinogen decreases risk of VTE and ischemic stroke. Our hypothesis for γ' fibrinogen was confirmed in our MR results and is most likely explained by γ' fibrinogen's anticoagulant properties. We also hypothesized that increasing total fibrinogen levels increases risk of VTE and ischemic stroke subtypes. The results from our MR indeed indicated such an effect on small vessel disease, but it also indicated a protective effect of total fibrinogen levels on VTE and cardioembolic stroke.

Strengths & Limitations

We used the largest GWAS of fibrinogen, VTE, and ischemic stroke, so we were well-powered to find effects (de Vries et al., 2016; Germain et al., 2015; Malik et al., 2018). Even though the γ' fibrinogen GWAS was relatively small (**Table 1**), we still were able to detect an effect and received significant results ($p < 0.05$) in many of our analyses. By using information across multiple variants, instead of a single genetic instrument, we further increased our power, and reduced the chances that a single genetic variant with strong pleiotropic effect is driving our results. Multiple variants in our genetic instruments came from the fibrinogen gene cluster. Because these genes encode for fibrinogen, it is unlikely these variants have pleiotropic effects that do not involve fibrinogen (Omarova et al., 2013). However, we also used many sensitivity analyses to account for potential pleiotropy.

Unfortunately, we do not know which variants are pleiotropic or the extent to which they are contributing to the effect estimate. We therefore do not know which of our sensitivity analyses is most appropriate. However, the consistency across the sensitivity analyses, present in many of our analyses (see **Appendix C and E**), suggest that if pleiotropy is present, it is either minimal or affects all sensitivity analyses similarly. The latter is unlikely since each sensitivity analyses is robust to different kinds of pleiotropy. Another limitation is that effect estimates were log transformed in the GWAS summary statistics we used for γ' fibrinogen and total fibrinogen levels. Since it is not as straightforward to transform them back, interpretation of the causal effect sizes results is difficult.

Next steps

Alternative explanations are needed to clarify why we observed a protective effect in our MR of genetically determined total fibrinogen levels on VTE, and cardioembolic stroke. Because γ' fibrinogen is a part of total fibrinogen levels, we first need to assess if the protective association of total fibrinogen levels is mediated by γ' fibrinogen levels. Additionally, because of fibrinogen's role in inflammation, (Davalos & Akassoglou, 2012) we also need to assess whether the associations are confounded by pleiotropy involving C-reactive protein (CRP). We plan to use multivariable MR to adjust total fibrinogen levels for γ' fibrinogen and CRP levels, separately, and see if a protective effect remains. Nevertheless, if pleiotropic effects on γ' fibrinogen and CRP levels explain our results it is unclear why our sensitivity analyses showed such consistent effects.

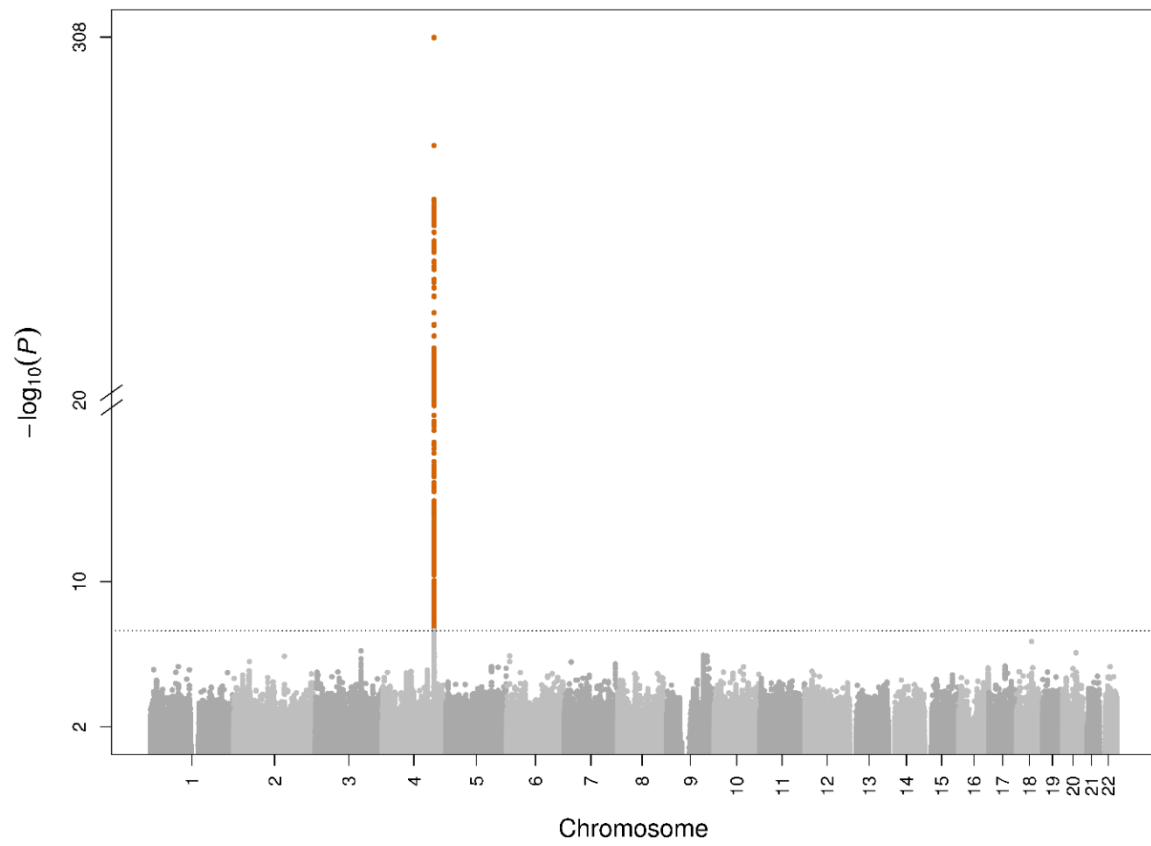
The protective association of total fibrinogen levels with VTE and cardioembolic stroke could instead be explained by an effect of fibrinogen on clot stabilization. Cardioembolic stroke and pulmonary embolism, which is part of the VTE definition, both involve embolism, in which part of the blood clot breaks off and travel to smaller blood vessels. Higher levels of total fibrinogen may increase clot stabilization, thereby preventing the events that result in pulmonary embolism and cardioembolic stroke (Wolberg et al., 2003). To determine whether this is the case, we will attempt to perform separate MRs for pulmonary embolism and deep vein thrombosis. We expect the former to be affected by clot stabilization but not the latter. However, the INVENT Consortium did not analyze these outcomes separately (only jointly as VTE), and we will thus need to identify an additional data source (Germain et al., 2015).

γ' fibrinogen's protective effect on VTE, cardioembolic stroke, and large artery stroke can pave the way for alternative therapies to be produced, other than typical anticoagulant medications that have significant risks. However, what remains unclear is whether increased absolute levels of γ' fibrinogen or increased ratio of γ' fibrinogen to total fibrinogen levels are more important in reducing risk of these outcomes (Uitte de Willige et al., 2005). To assess this, a GWAS of γ' fibrinogen to total fibrinogen ratio should be performed and then used in MR analyses, so that we can deduce which has the greater impact. Unfortunately, γ' fibrinogen and total fibrinogen levels were not assessed in the same visit among ARIC study participants, so we could not use the ARIC study to perform this additional analysis. More in vivo animal studies should be performed to assess the efficacy and safety of increasing γ' fibrinogen levels (or the γ' fibrinogen to total fibrinogen ratio) before considering its potential as an alternative for anticoagulant medications.

Finally, as total fibrinogen levels have already been implicated in causing coronary heart disease (Ward-Caviness et al., 2018) our MR suggests the need to evaluate γ' fibrinogen levels as well, in assessing causal effect on cardiovascular outcomes, such as coronary heart disease.

APPENDICES

Appendix A: Manhattan plot for the genome-wide association of γ' fibrinogen levels.



Appendix B: Causal effect estimates produced by individual genetic instruments for γ' fibrinogen

Figure 1: γ' fibrinogen levels on VTE

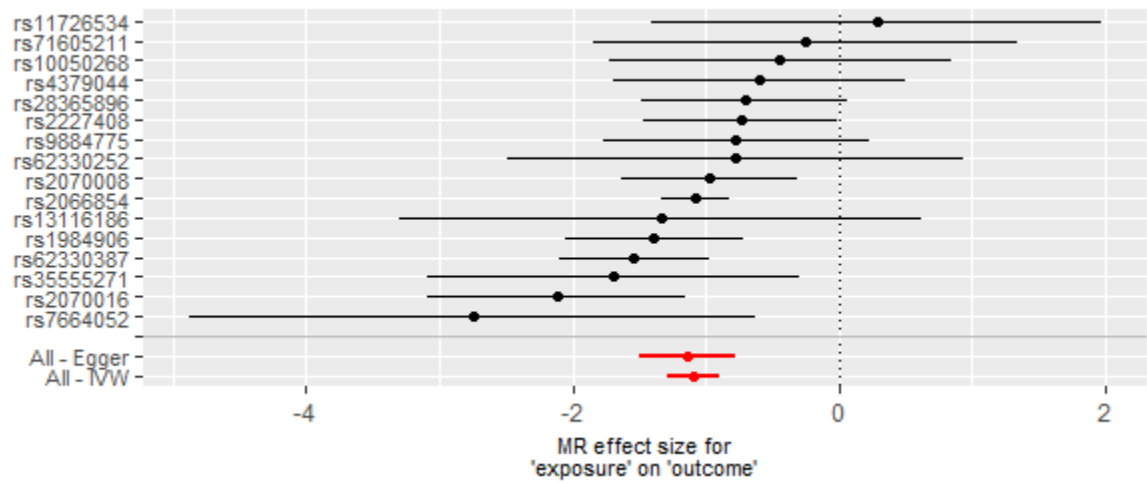


Figure 2: γ' fibrinogen on cardioembolic stroke

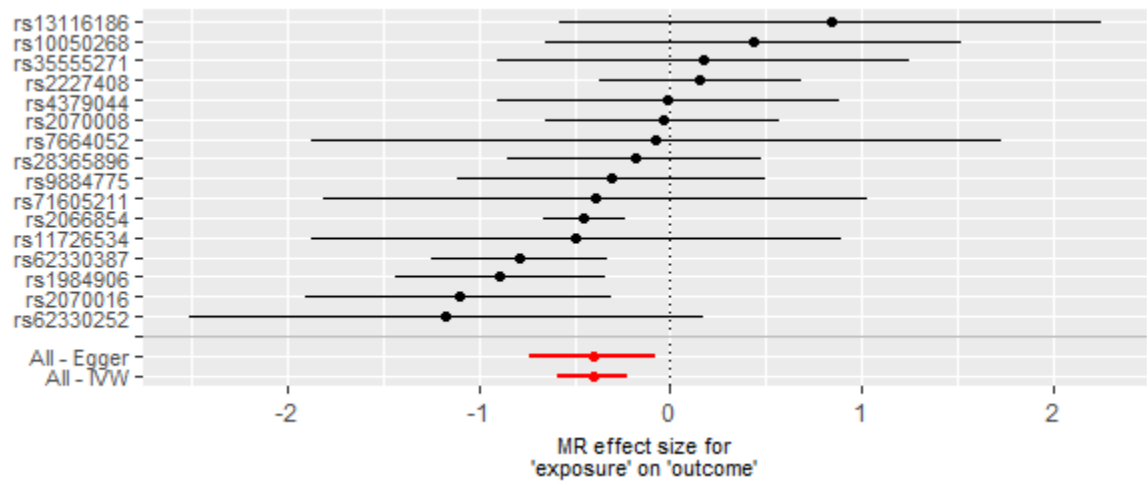


Figure 3: γ' fibrinogen on large artery stroke

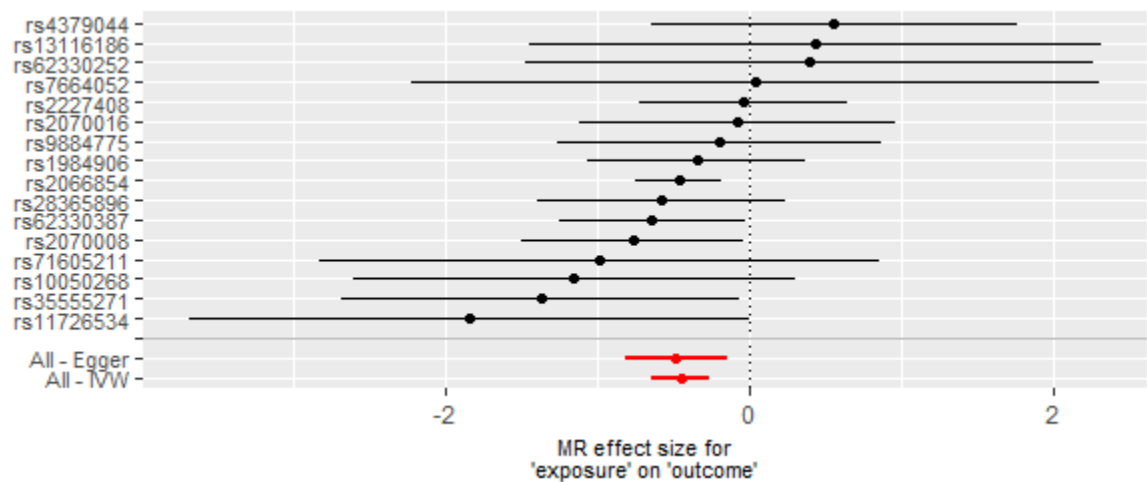


Figure 4: γ' fibrinogen on small vessel stroke

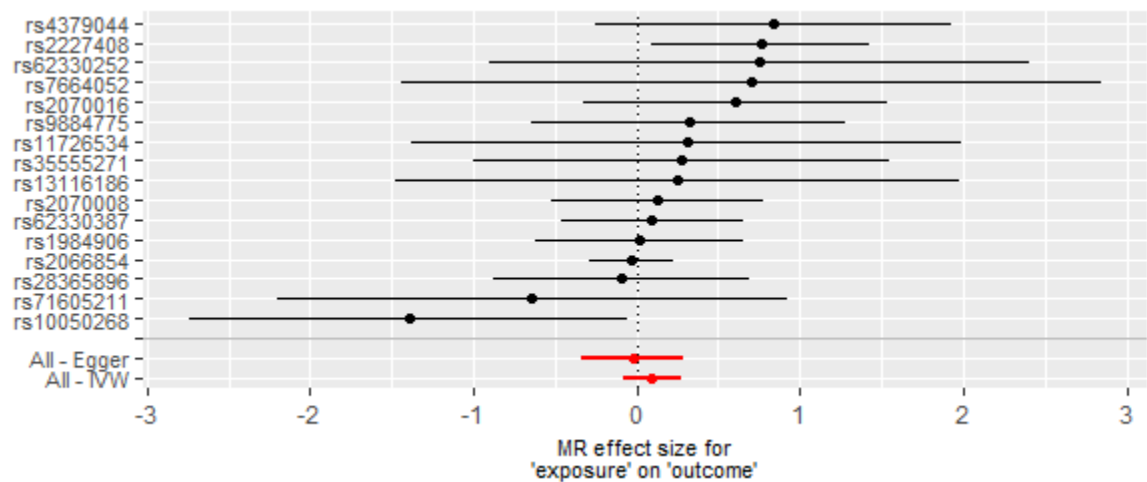
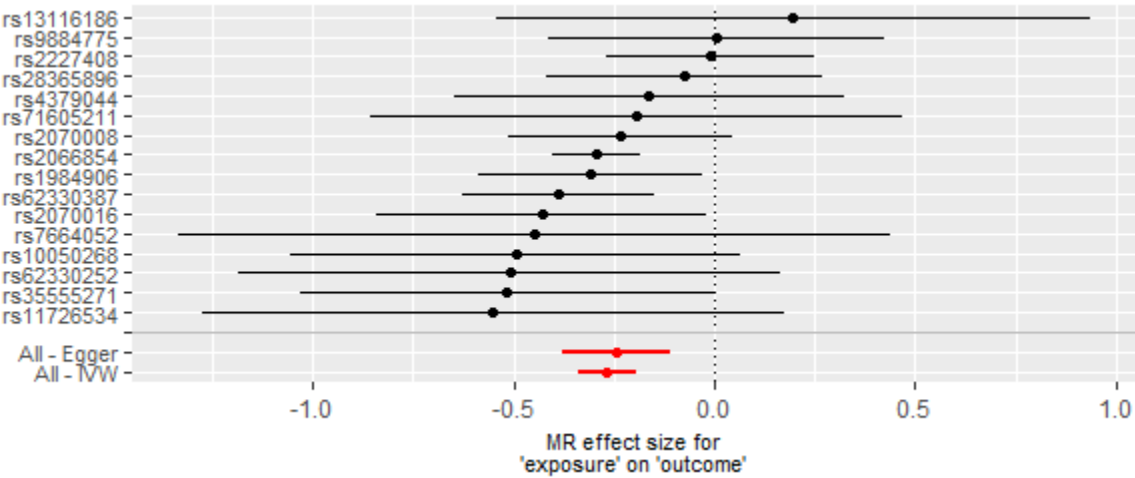


Figure 5: γ' fibrinogen on any ischemic stroke



Appendix C: Sensitivity analyses for Mendelian randomization of γ' fibrinogen levels

Figure 1: γ' fibrinogen levels on venous thromboembolism

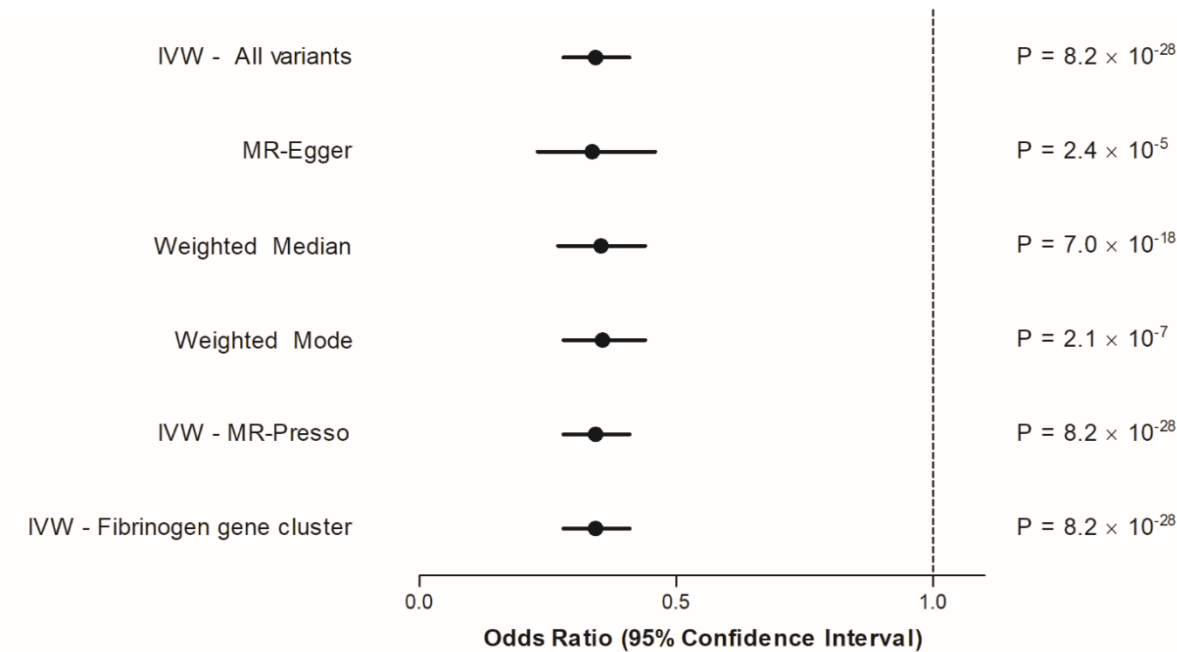


Figure 2: γ' fibrinogen levels on cardioembolic stroke

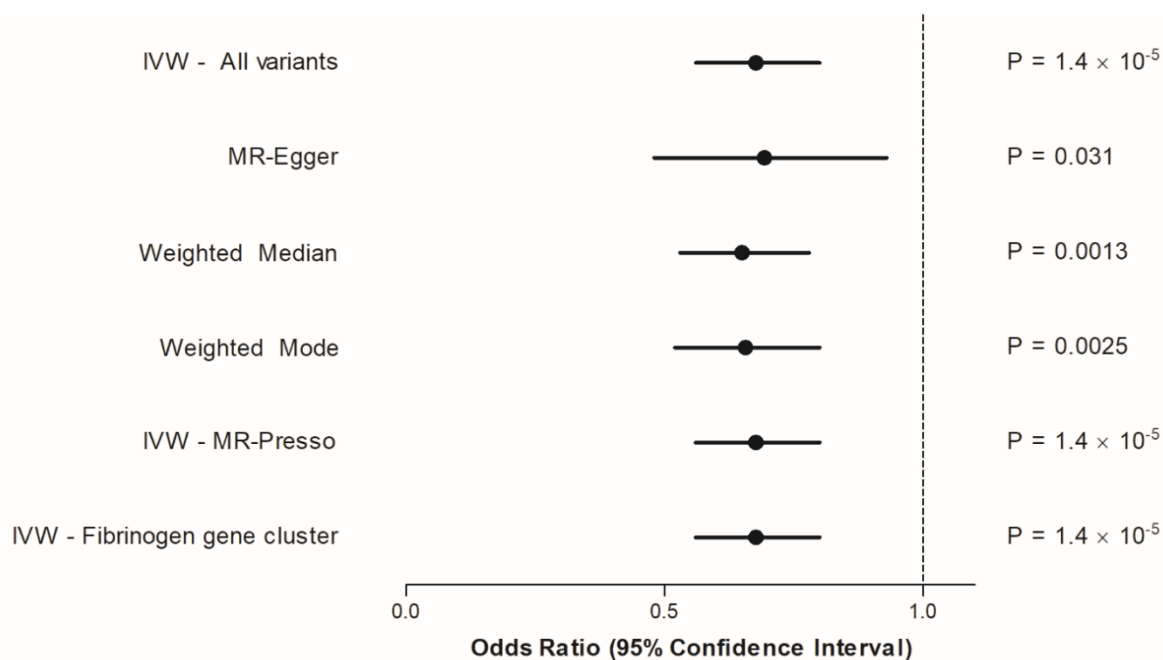


Figure 3: γ' fibrinogen levels on large artery stroke

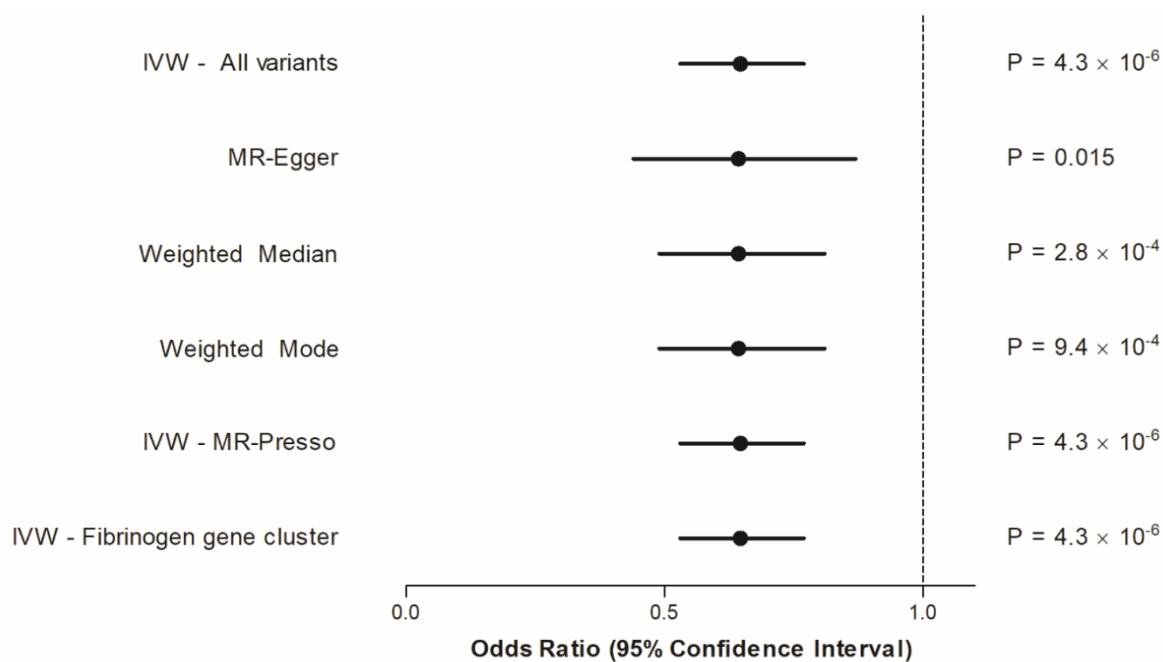


Figure 4: γ' fibrinogen levels on small vessel stroke

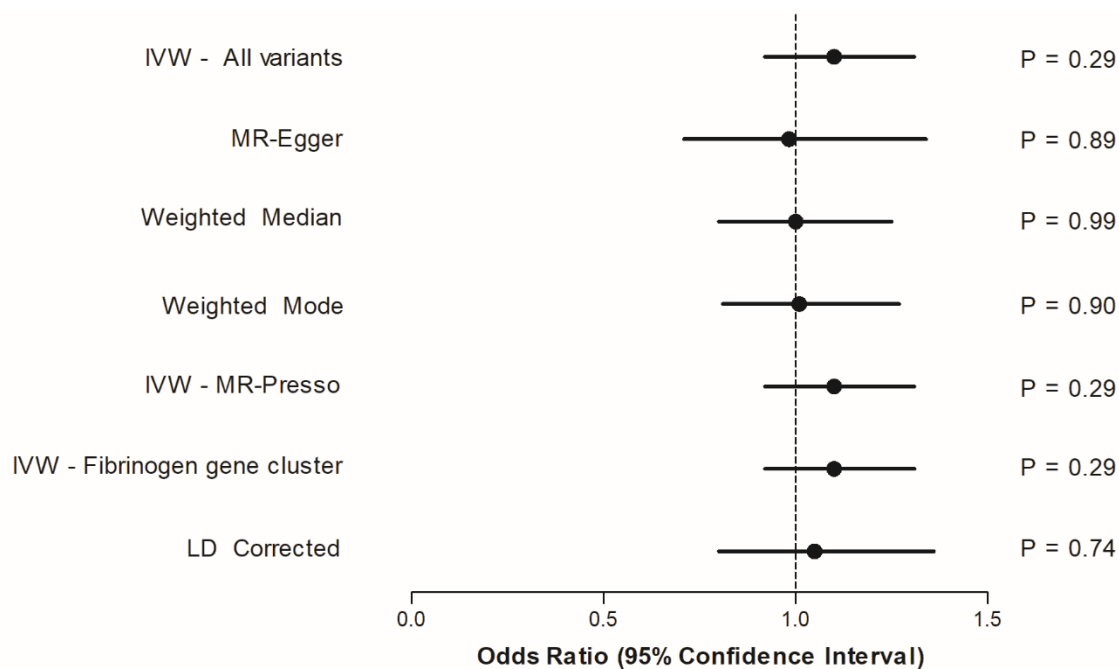
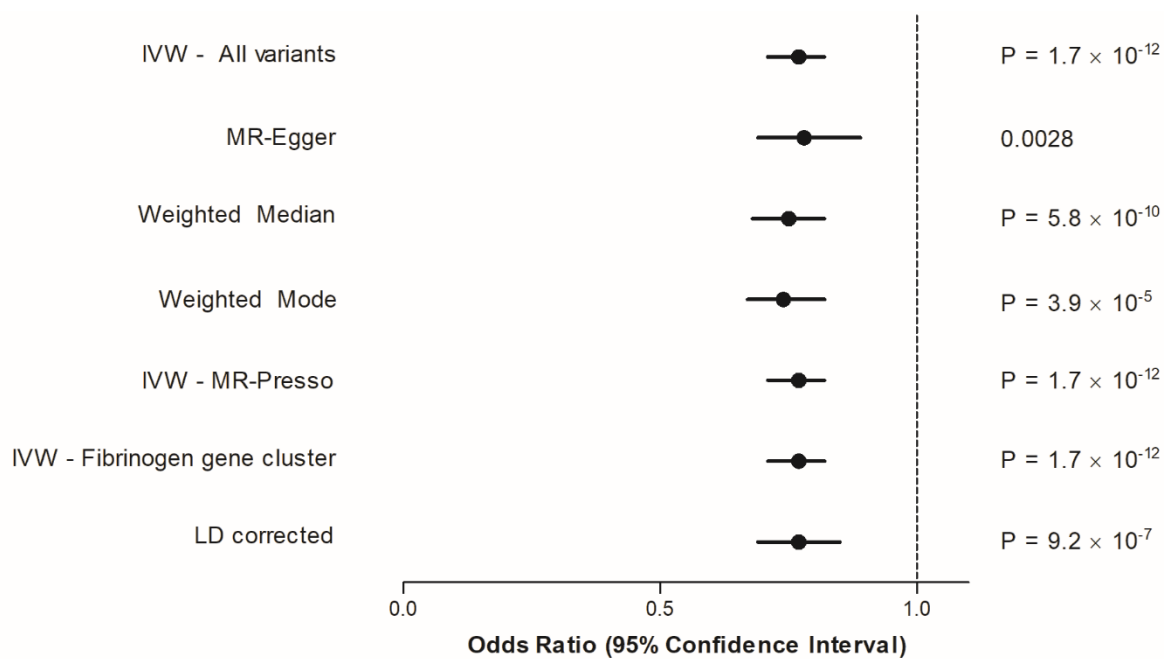


Figure 5: γ' fibrinogen levels on any ischemic stroke



Appendix D: Causal effect estimates produced by individual genetic instruments for total fibrinogen levels.

Figure 1: Total fibrinogen levels on venous thromboembolism

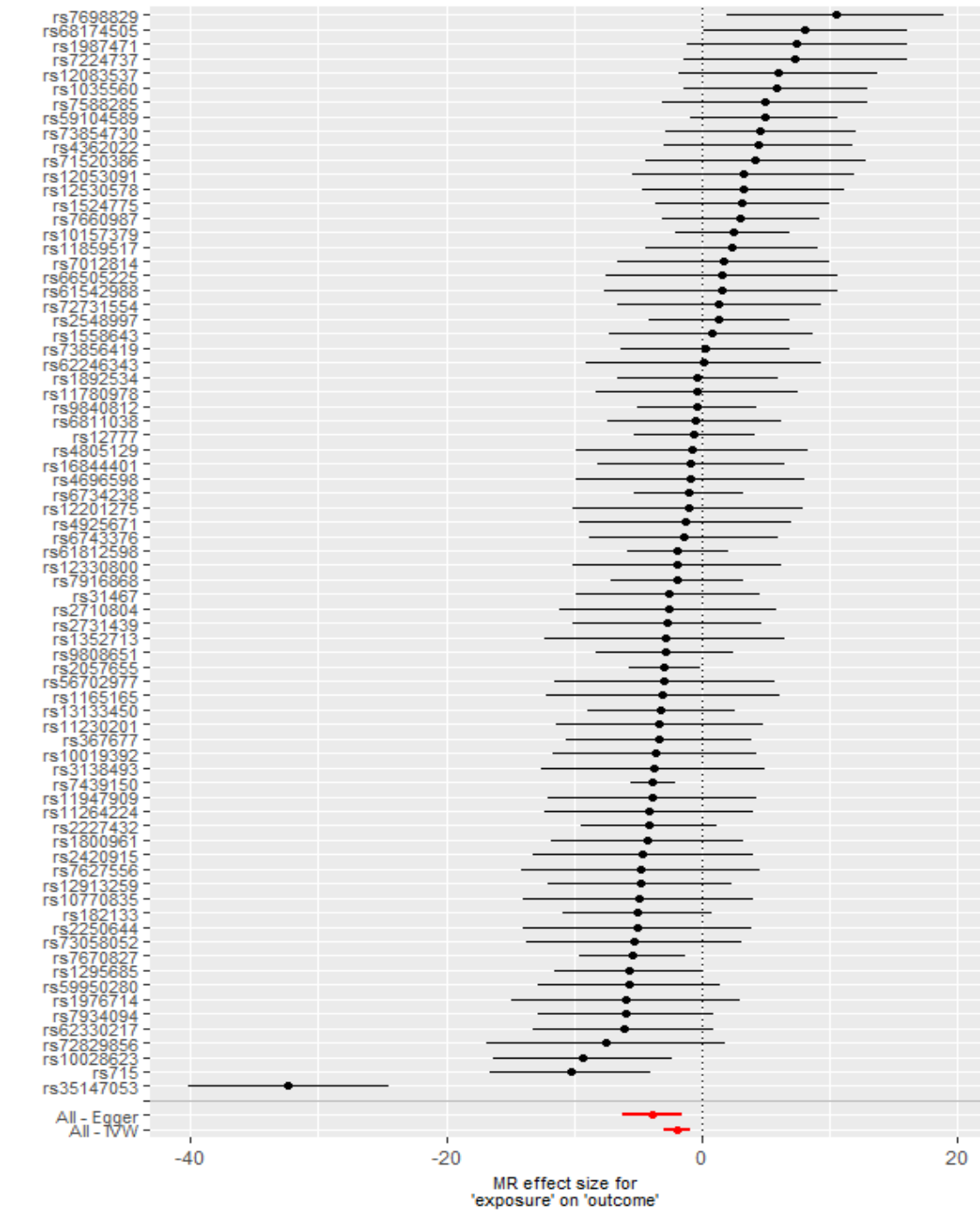


Figure 2: Total fibrinogen levels on cardioembolic stroke

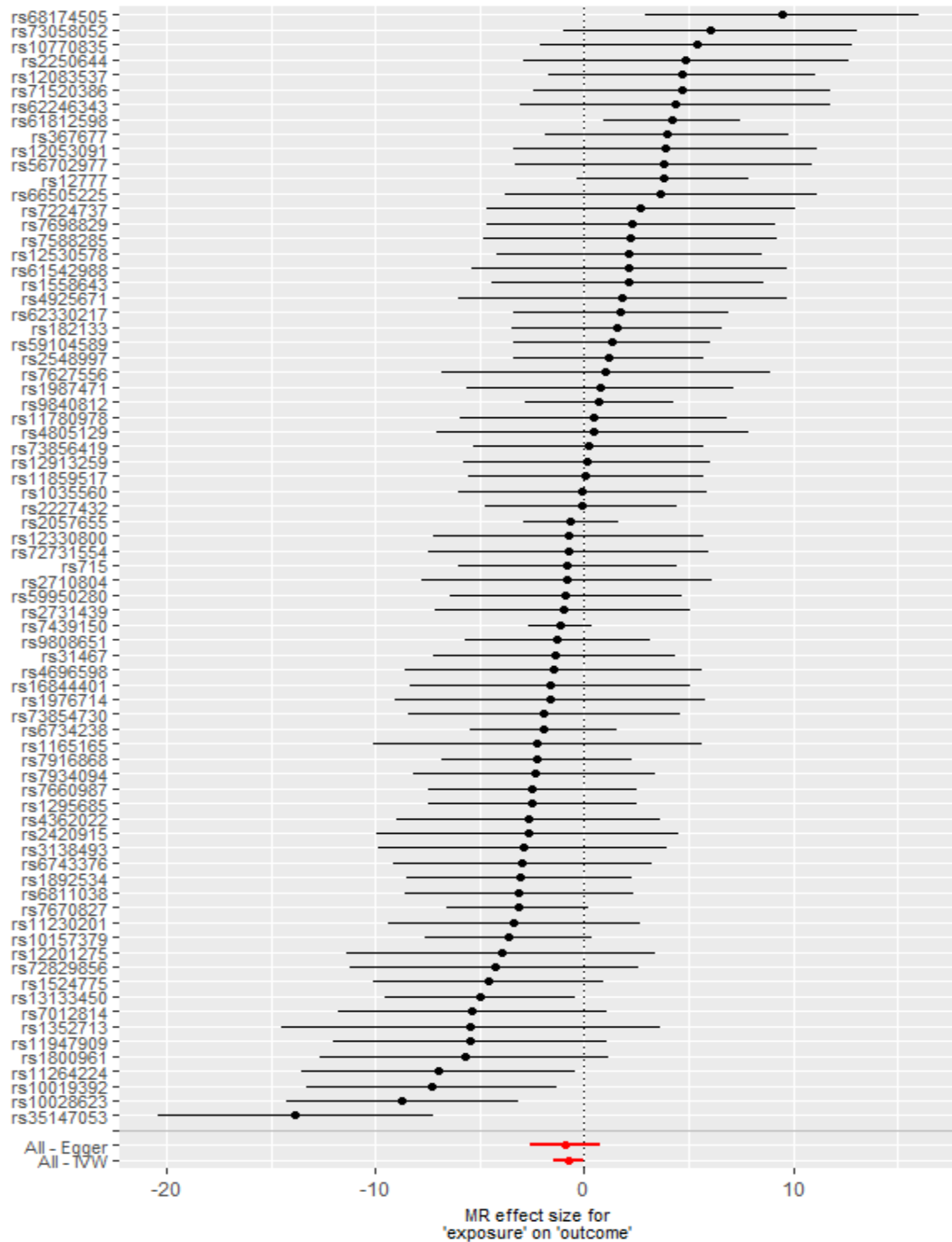


Figure 3: Total fibrinogen levels on large artery stroke

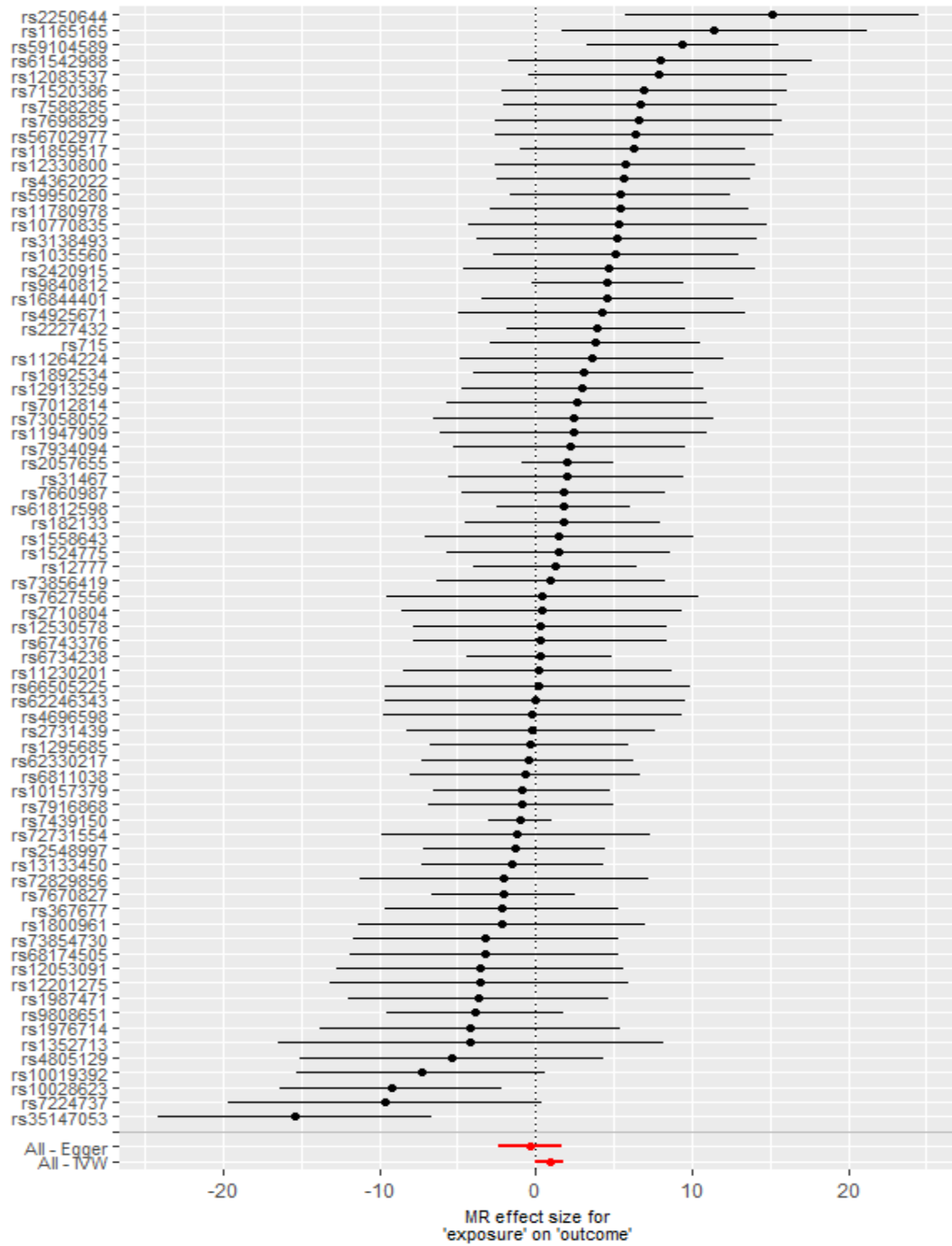


Figure 4: Total fibrinogen levels on small vessel stroke

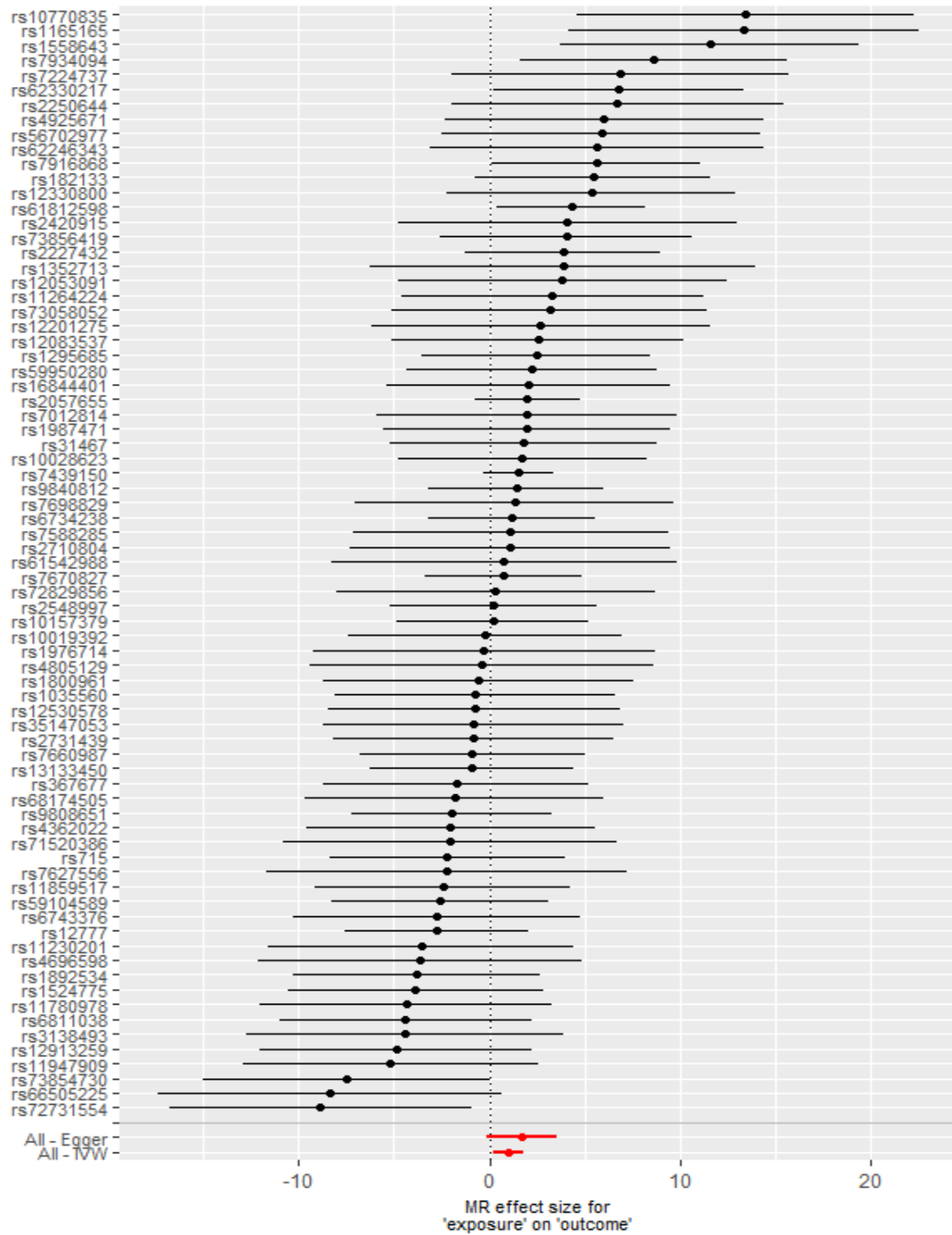
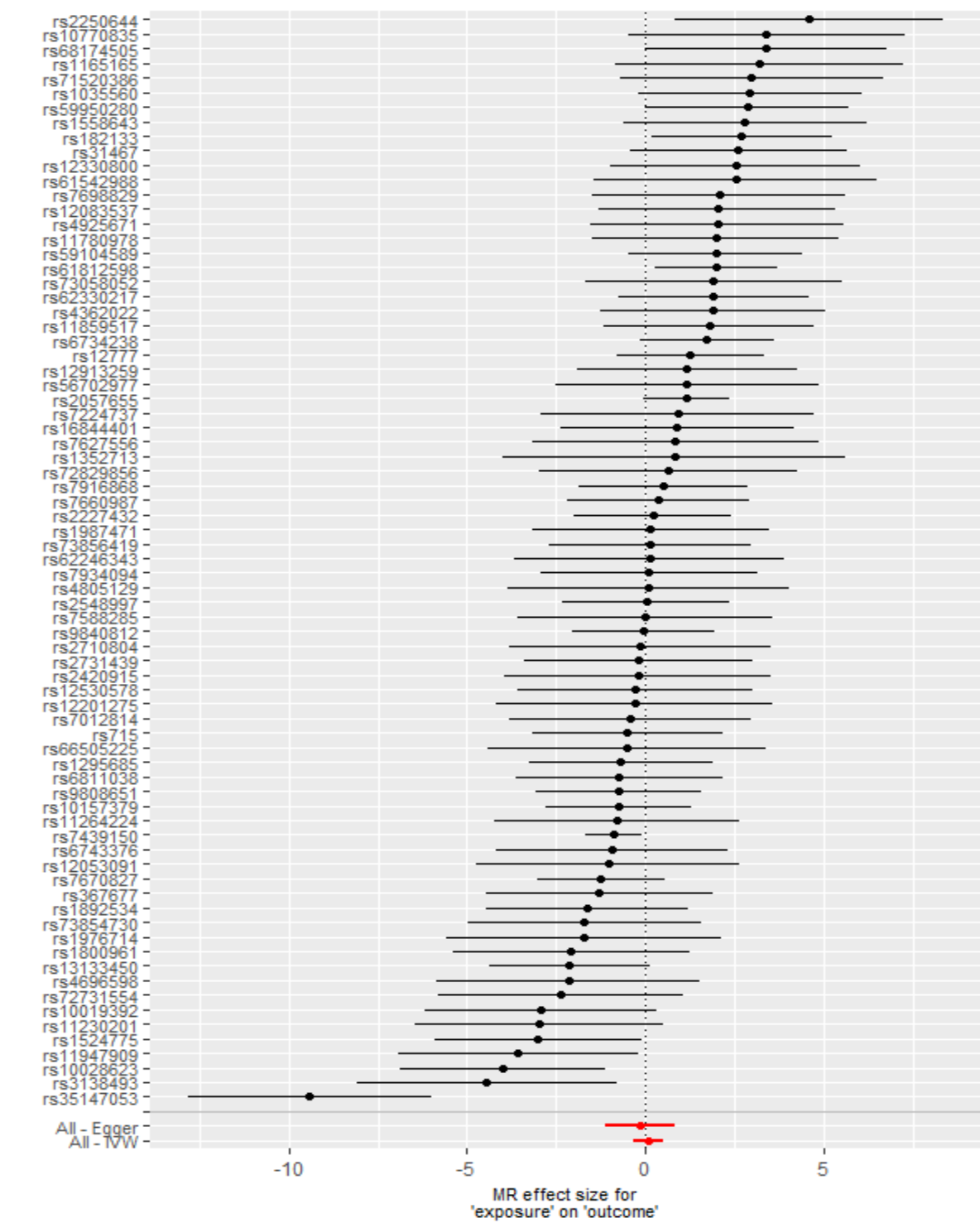


Figure 5: Total fibrinogen levels on any ischemic stroke



Appendix E: Sensitivity analyses for Mendelian randomization of total fibrinogen levels

Figure 1: Total fibrinogen levels on venous thromboembolism

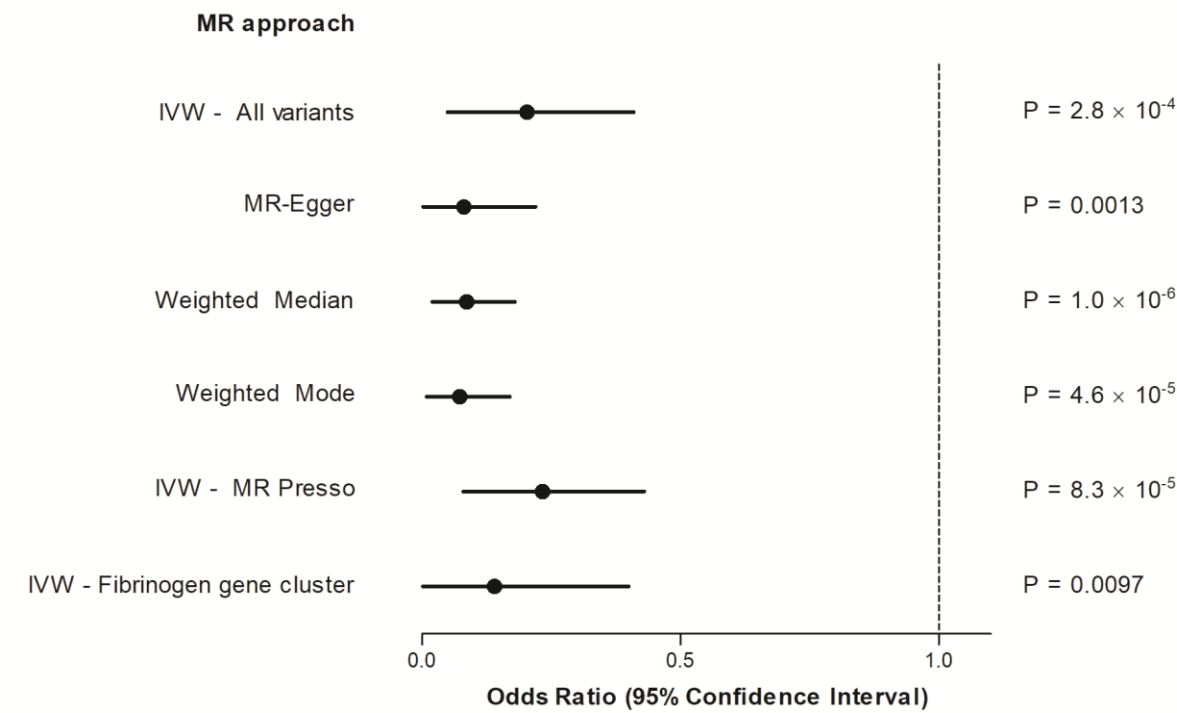


Figure 2: Total fibrinogen levels on cardioembolic stroke

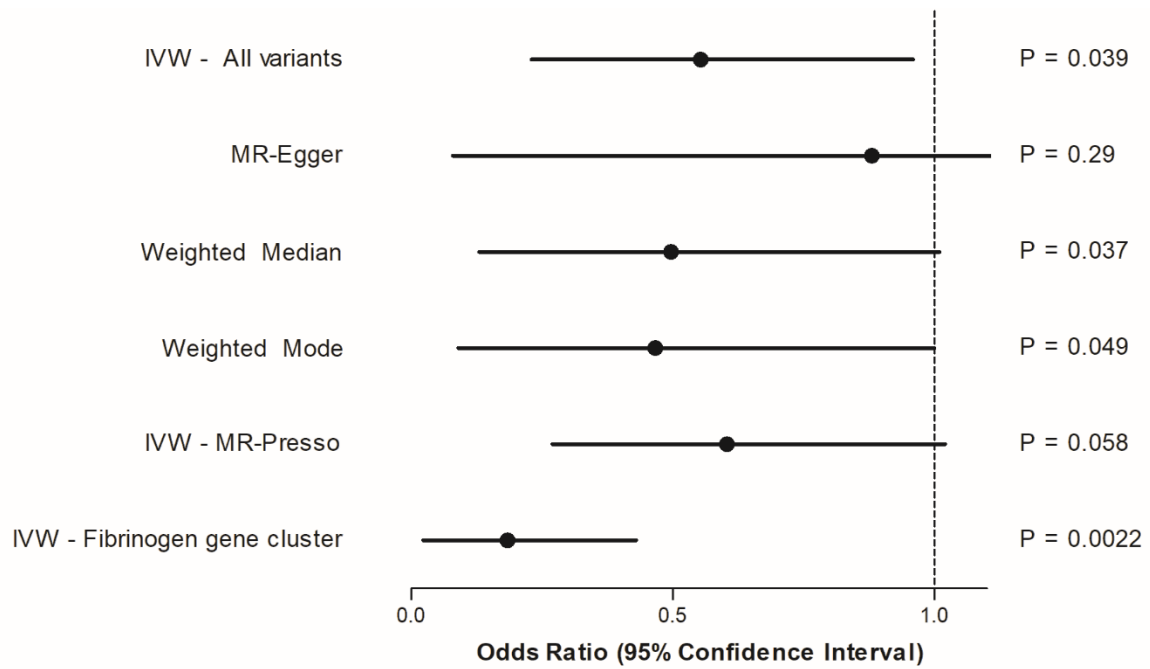


Figure 3: Total fibrinogen levels on large artery stroke

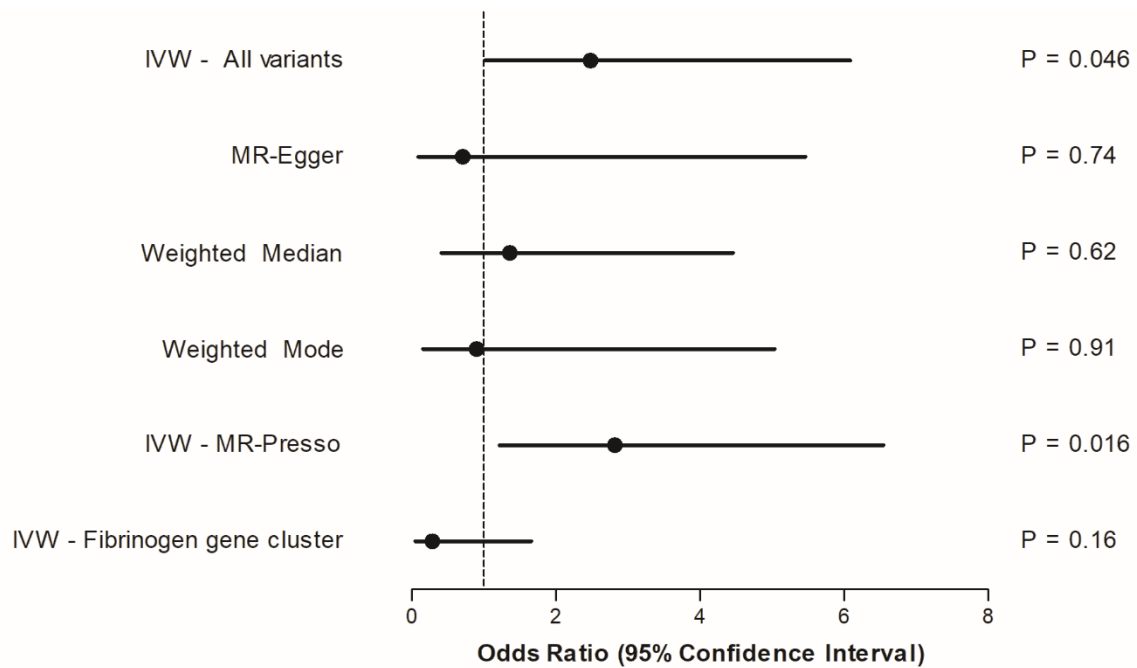


Figure 4: Total fibrinogen levels on small vessel stroke

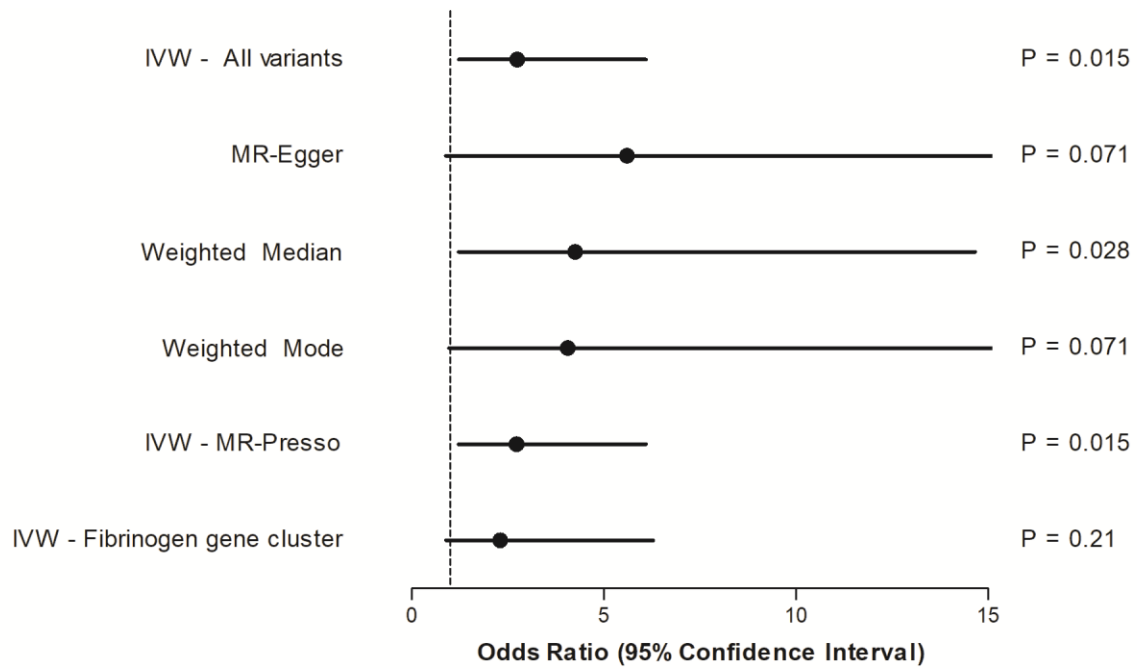
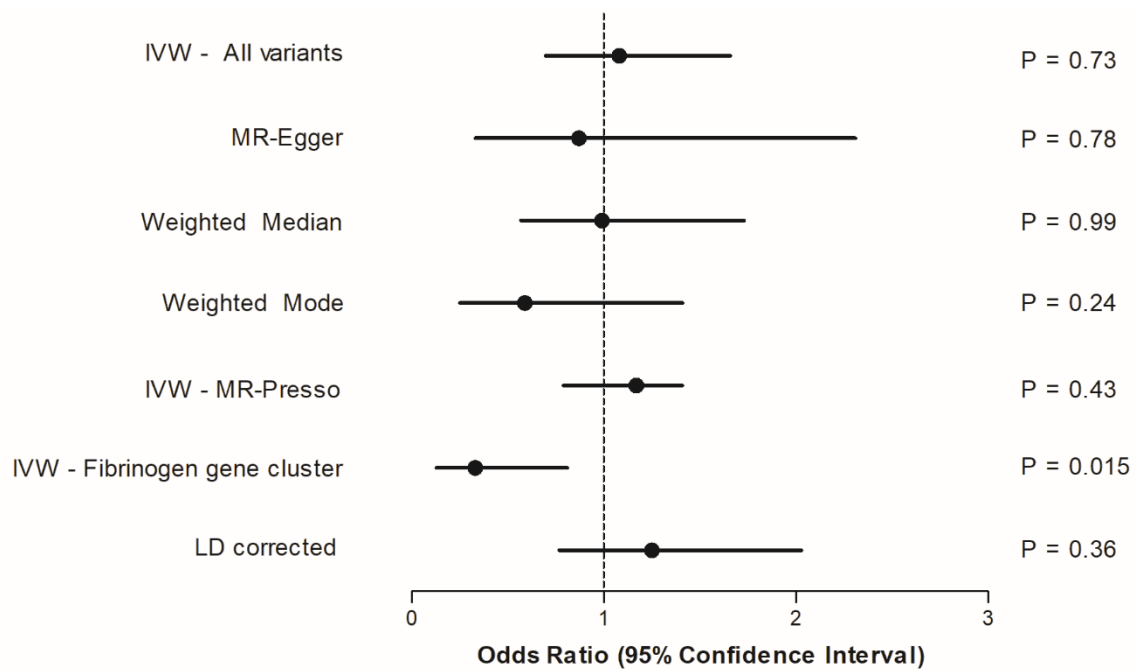


Figure 5: Total fibrinogen levels on any ischemic stroke



REFERENCES

- 1000 Genomes Project Consortium, Abecasis, G. R., Auton, A., Brooks, L. D., DePristo, M. A., Durbin, R. M., . . . McVean, G. A. (2012). An integrated map of genetic variation from 1,092 human genomes. *Nature*, *491*(7422), 56-65. doi:10.1038/nature11632 [doi]
- Adams, H. P., Jr, Bendixen, B. H., Kappelle, L. J., Biller, J., Love, B. B., Gordon, D. L., & Marsh, E. E., 3rd. (1993). Classification of subtype of acute ischemic stroke. definitions for use in a multicenter clinical trial. TOAST. trial of org 10172 in acute stroke treatment. *Stroke*, *24*(1), 35-41.
- Appiah, D., Heckbert, S. R., Cushman, M., Psaty, B. M., & Folsom, A. R. (2016). Lack of association of plasma gamma prime (gamma') fibrinogen with incident cardiovascular disease. *Thrombosis Research*, *143*, 50-52. doi:10.1016/j.thromres.2016.04.023 [doi]
- Appiah, D., Schreiner, P. J., MacLehose, R. F., & Folsom, A. R. (2015). Association of plasma gamma' fibrinogen with incident cardiovascular disease: The atherosclerosis risk in communities (ARIC) study. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *35*(12), 2700-2706. doi:10.1161/ATVBAHA.115.306284 [doi]
- Banerjee, C., & Turan, T. N. (2017). Large artery atherosclerosis: Extracranial and intracranial. *Seminars in Neurology*, *37*(3), 307-315. doi:10.1055/s-0037-1603588 [doi]

- Bowden, J., Davey Smith, G., & Burgess, S. (2015). Mendelian randomization with invalid instruments: Effect estimation and bias detection through egger regression. *International Journal of Epidemiology*, 44(2), 512-525. doi:10.1093/ije/dyv080 [doi]
- Bowden, J., Davey Smith, G., Haycock, P. C., & Burgess, S. (2016). Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genetic Epidemiology*, 40(4), 304-314. doi:10.1002/gepi.21965 [doi]
- Burgess, S., Butterworth, A., & Thompson, S. G. (2013). Mendelian randomization analysis with multiple genetic variants using summarized data. *Genetic Epidemiology*, 37(7), 658-665. doi:10.1002/gepi.21758 [doi]
- Burgess, S., & Thompson, S. G. (2015). Multivariable mendelian randomization: The use of pleiotropic genetic variants to estimate causal effects. *American Journal of Epidemiology*, 181(4), 251-260. doi:10.1093/aje/kwu283 [doi]
- Chanda, P., Huang, H., Arking, D. E., & Bader, J. S. (2013). Fast association tests for genes with FAST. *PloS One*, 8(7), e68585. doi:10.1371/journal.pone.0068585 [doi]
- Cheung, E. Y., Uitte de Willige, S., Vos, H. L., Leebeek, F. W., Dippel, D. W., Bertina, R. M., & de Maat, M. P. (2008). Fibrinogen gamma' in ischemic stroke: A case-control study. *Stroke*, 39(3), 1033-1035. doi:10.1161/STROKEAHA.107.495499 [doi]
- Chuang, S. Y., Bai, C. H., Chen, W. H., Lien, L. M., & Pan, W. H. (2009). Fibrinogen independently predicts the development of ischemic stroke in a taiwanese population: CVDFACTS study. *Stroke*, 40(5), 1578-1584. doi:10.1161/STROKEAHA.108.540492 [doi]

- Chung, D. W., & Davie, E. W. (1984). Gamma and gamma' chains of human fibrinogen are produced by alternative mRNA processing. *Biochemistry*, 23(18), 4232-4236.
- Collet, J. P., Nagaswami, C., Farrell, D. H., Montalescot, G., & Weisel, J. W. (2004). Influence of gamma' fibrinogen splice variant on fibrin physical properties and fibrinolysis rate. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 24(2), 382-386.
doi:10.1161/01.ATV.0000109748.77727.3e [doi]
- Davalos, D., & Akassoglou, K. (2012). Fibrinogen as a key regulator of inflammation in disease. *Seminars in Immunopathology*, 34(1), 43-62. doi:10.1007/s00281-011-0290-8 [doi]
- Davey Smith, G., & Hemani, G. (2014). Mendelian randomization: Genetic anchors for causal inference in epidemiological studies. *Human Molecular Genetics*, 23(R1), R89-98.
doi:10.1093/hmg/ddu328 [doi]
- de Bosch, N. B., Mosesson, M. W., Ruiz-Saez, A., Echenagucia, M., & Rodriguez-Lemoin, A. (2002). Inhibition of thrombin generation in plasma by fibrin formation (antithrombin I). *Thrombosis and Haemostasis*, 88(2), 253-258.
- de Vries, P. S., Chasman, D. I., Sabater-Lleal, M., Chen, M. H., Huffman, J. E., Steri, M., . . . Dehghan, A. (2016). A meta-analysis of 120 246 individuals identifies 18 new loci for fibrinogen concentration. *Human Molecular Genetics*, 25(2), 358-370. doi:10.1093/hmg/ddv454 [doi]
- Farrell, D. H. (2012). Gamma' fibrinogen as a novel marker of thrombotic disease. *Clinical Chemistry and Laboratory Medicine*, 50(11), 1903-1909. doi:10.1515/cclm-2012-0005 [doi]

- Fibrinogen Studies Collaboration, Danesh, J., Lewington, S., Thompson, S. G., Lowe, G. D., Collins, R., . . . Wood, A. (2005). Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: An individual participant meta-analysis. *Jama*, 294(14), 1799-1809. doi:294/14/1799 [pii]
- Folsom, A. R., Tang, W., George, K. M., Heckbert, S. R., MacLehose, R. F., Cushman, M., & Pankow, J. S. (2016). Prospective study of gamma' fibrinogen and incident venous thromboembolism: The longitudinal investigation of thromboembolism etiology (LITE). *Thrombosis Research*, 139, 44-49. doi:10.1016/j.thromres.2016.01.008 [doi]
- Germain, M., Chasman, D. I., de Haan, H., Tang, W., Lindstrom, S., Weng, L. C., . . . Morange, P. E. (2015). Meta-analysis of 65,734 individuals identifies TSPAN15 and SLC44A2 as two susceptibility loci for venous thromboembolism. *American Journal of Human Genetics*, 96(4), 532-542. doi:10.1016/j.ajhg.2015.01.019 [doi]
- Hartwig, F. P., Davey Smith, G., & Bowden, J. (2017). Robust inference in summary data mendelian randomization via the zero modal pleiotropy assumption. *International Journal of Epidemiology*, 46(6), 1985-1998. doi:10.1093/ije/dyx102 [doi]
- Haycock, P. C., Burgess, S., Wade, K. H., Bowden, J., Relton, C., & Davey Smith, G. (2016). Best (but oft-forgotten) practices: The design, analysis, and interpretation of mendelian randomization studies. *The American Journal of Clinical Nutrition*, 103(4), 965-978. doi:ajcn.115.118216 [pii]

Howie, B., Fuchsberger, C., Stephens, M., Marchini, J., & Abecasis, G. R. (2012). Fast and accurate genotype imputation in genome-wide association studies through pre-phasing. *Nature Genetics*, 44(8), 955-959. doi:10.1038/ng.2354 [doi]

Howie, B., Marchini, J., & Stephens, M. (2011). Genotype imputation with thousands of genomes. *G3 (Bethesda, Md.)*, 1(6), 457-470. doi:10.1534/g3.111.001198 [doi]

Hunt, B. J., Parmar, K., Horspool, K., Shephard, N., Nelson-Piercy, C., Goodacre, S., & DiPEP research group. (2018). The DiPEP (diagnosis of PE in pregnancy) biomarker study: An observational cohort study augmented with additional cases to determine the diagnostic utility of biomarkers for suspected venous thromboembolism during pregnancy and puerperium. *British Journal of Haematology*, 180(5), 694-704. doi:10.1111/bjh.15102 [doi]

Khan, U., Porteous, L., Hassan, A., & Markus, H. S. (2007). Risk factor profile of cerebral small vessel disease and its subtypes. *Journal of Neurology, Neurosurgery, and Psychiatry*, 78(7), 702-706. doi:jnnp.2006.103549 [pii]

Kim, J. M., Jung, K. H., Park, K. H., Lee, S. T., Chu, K., & Roh, J. K. (2013). Clinical manifestation of cancer related stroke: Retrospective case-control study. *Journal of Neuro-Oncology*, 111(3), 295-301. doi:10.1007/s11060-012-1011-4 [doi]

Koster, T., Rosendaal, F. R., Reitsma, P. H., van der Velden, P. A., Briet, E., & Vandenbroucke, J. P. (1994). Factor VII and fibrinogen levels as risk factors for venous thrombosis. A case-control study of plasma levels and DNA polymorphisms--the leiden thrombophilia study (LETS). *Thrombosis and Haemostasis*, 71(6), 719-722.

- Lancellotti, S., Rutella, S., De Filippis, V., Pozzi, N., Rocca, B., & De Cristofaro, R. (2008). Fibrinogen-elongated gamma chain inhibits thrombin-induced platelet response, hindering the interaction with different receptors. *The Journal of Biological Chemistry*, 283(44), 30193-30204. doi:10.1074/jbc.M803659200 [doi]
- Lovely, R. S., Boshkov, L. K., Marzec, U. M., Hanson, S. R., & Farrell, D. H. (2007). Fibrinogen gamma' chain carboxy terminal peptide selectively inhibits the intrinsic coagulation pathway. *British Journal of Haematology*, 139(3), 494-503. doi:BJH6825 [pii]
- Malik, R., Chauhan, G., Traylor, M., Sargurupremraj, M., Okada, Y., Mishra, A., . . . MEGASTROKE Consortium:. (2018). Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nature Genetics*, 50(4), 524-537. doi:10.1038/s41588-018-0058-3 [doi]
- Mosesson, M. W. (2003). Fibrinogen gamma chain functions. *Journal of Thrombosis and Haemostasis : JTH*, 1(2), 231-238. doi:63 [pii]
- Mosesson, M. W. (2005). Fibrinogen and fibrin structure and functions. *Journal of Thrombosis and Haemostasis : JTH*, 3(8), 1894-1904. doi:JTH1365 [pii]
- O'Carroll, C. B., & Barrett, K. M. (2017). Cardioembolic stroke. *Continuum (Minneapolis, Minn.)*, 23(1, Cerebrovascular Disease), 111-132. doi:10.1212/CON.0000000000000419 [doi]
- Omarova, F., Uitte De Willige, S., Ariens, R. A., Rosing, J., Bertina, R. M., & Castoldi, E. (2013). Inhibition of thrombin-mediated factor V activation contributes to the anticoagulant activity of

fibrinogen gamma'. *Journal of Thrombosis and Haemostasis : JTH*, 11(9), 1669-1678.

doi:10.1111/jth.12354 [doi]

Patel, K., Fasanya, A., Yadav, S., Joshi, A. A., Singh, A. C., & DuMont, T. (2017). Pathogenesis and epidemiology of venous thromboembolic disease. *Critical Care Nursing Quarterly*, 40(3), 191-200. doi:10.1097/CNQ.0000000000000158 [doi]

Payne, A. B., Miller, C. H., Hooper, W. C., Lally, C., & Austin, H. D. (2014). High factor VIII, von willebrand factor, and fibrinogen levels and risk of venous thromboembolism in blacks and whites. *Ethnicity & Disease*, 24(2), 169-174.

Sidelmann, J. J., Gram, J., Jespersen, J., & Kluft, C. (2000). Fibrin clot formation and lysis: Basic mechanisms. *Seminars in Thrombosis and Hemostasis*, 26(6), 605-618. doi:10.1055/s-2000-13216 [doi]

Siebenlist, K. R., Mosesson, M. W., Hernandez, I., Bush, L. A., Di Cera, E., Shainoff, J. R., . . . Stojanovic, L. (2005). Studies on the basis for the properties of fibrin produced from fibrinogen-containing gamma' chains. *Blood*, 106(8), 2730-2736. doi:2005-01-0240 [pii]

Siegerink, B., Rosendaal, F. R., & Algra, A. (2009). Genetic variation in fibrinogen; its relationship to fibrinogen levels and the risk of myocardial infarction and ischemic stroke. *Journal of Thrombosis and Haemostasis : JTH*, 7(3), 385-390. doi:10.1111/j.1538-7836.2008.03266.x [doi]

Traylor, M., Bevan, S., Baron, J. C., Hassan, A., Lewis, C. M., & Markus, H. S. (2015). Genetic architecture of lacunar stroke. *Stroke*, 46(9), 2407-2412. doi:10.1161/STROKEAHA.115.009485 [doi]

- Tsai, A. W., Cushman, M., Rosamond, W. D., Heckbert, S. R., Tracy, R. P., Aleksic, N., & Folsom, A. R. (2002). Coagulation factors, inflammation markers, and venous thromboembolism: The longitudinal investigation of thromboembolism etiology (LITE). *The American Journal of Medicine*, 113(8), 636-642. doi:S0002934302013451 [pii]
- Uitte de Willige, S., de Visser, M. C., Houwing-Duistermaat, J. J., Rosendaal, F. R., Vos, H. L., & Bertina, R. M. (2005). Genetic variation in the fibrinogen gamma gene increases the risk for deep venous thrombosis by reducing plasma fibrinogen gamma' levels. *Blood*, 106(13), 4176-4183. doi:2005-05-2180 [pii]
- van den Herik, E. G., Cheung, E. Y., de Lau, L. M., den Hertog, H. M., Leebeek, F. W., Dippel, D. W., . . . de Maat, M. P. (2011). Gamma'/total fibrinogen ratio is associated with short-term outcome in ischaemic stroke. *Thrombosis and Haemostasis*, 105(3), 430-434. doi:10.1160/TH10-09-0569 [doi]
- Ward-Caviness, C. K., de Vries, P. S., Wiggins, K. L., Huffman, J. E., Yanek, L. R., Bielak, L. F., . . . Morrison, A. C. (2018). Evaluation of the causal effect of fibrinogen on incident coronary heart disease via mendelian randomization [Abstract]. *BioRxiv* doi:<https://doi.org/10.1101/448381>
- Wolberg, A. S., Monroe, D. M., Roberts, H. R., & Hoffman, M. (2003). Elevated prothrombin results in clots with an altered fiber structure: A possible mechanism of the increased thrombotic risk. *Blood*, 101(8), 3008-3013. doi:10.1182/blood-2002-08-2527 [doi]
- Yamashita, Y., Wada, H., Nomura, H., Mizuno, T., Saito, K., Yamada, N., . . . Katayama, N. (2014). Elevated fibrin-related markers in patients with malignant diseases frequently associated with

disseminated intravascular coagulation and venous thromboembolism. *Internal Medicine (Tokyo, Japan)*, 53(5), 413-419. doi:DN/JST.JSTAGE/internalmedicine/53.1102 [pii]

Zhang, J., Yu, L., Yin, Y., Lu, Q., Lei, L., Xiao, J., . . . He, L. (2013). Association between two functional fibrinogen-related polymorphisms and ischemic stroke: A case-control study. *Genetic Testing and Molecular Biomarkers*, 17(11), 789-793. doi:10.1089/gtmb.2013.0131 [doi]