Stroke
Manuscript Submission and Peer Review System

https://doi.org/10.1161/STROKEAHA.119.026089

Title: Genetically determined risk of depression and functional outcome after ischemic stroke: Mendelian randomization study

Manuscript number: STROKE/2019/026089D

Author(s): Dipender Gill, Nicole James, Grace Monori, Erik Lorentzen, Israel Fernandez-Cadenas, Robin Lemmens, Vincent N Thijs, Natalia Rost, Rodney Scott, Graeme Hankey, Arne Lindgren, Christina Jern, and Jane Maguire

Group Authorship: International Stroke Genetics Consortium; Genetics of Ischaemic Stroke Functional Outcome network

Genetically determined risk of depression and functional outcome after ischemic stroke: Mendelian randomization study

Dipender Gill MD[1], Nicole E James BSc[2], Grace Monori BSc[2], Erik Lorentzen MSc[3], Israel Fernandez-Cadenas MSc PhD[4,5], Robin Lemmens MD PhD[6-8], Vincent Thijs MD PhD[9,10], Natalia S Rost MD PhD[11], Rodney Scott DSc PhD[12,13], Graeme J Hankey MD[14], Arne Lindgren MD PhD[15,16], Christina Jern MD PhD[17] and Jane M Maguire RN PhD[18-20]; on behalf of the International Stroke Genetics Consortium and the Genetics of Ischaemic Stroke Functional Outcome (GISCOME) network.

Affiliations

[1]Department of Biostatistics and Epidemiology, School of Public Health, Imperial College London, London, United Kingdom.

[2]Faculty of Medicine, Imperial College London, London, United Kingdom.

[3]Bioinformatics Core Facility, University of Gothenburg, Gothenburg, Sweden.

[4]Stroke Pharmacogenomics and Genetics, Institut d'investigació Biomedica de Sant Pau, Hospital de Sant Pau, Barcelona, Spain.

[5]Neurovascular Research Laboratory and Neurovascular Unit, Neurology and Medicine Departments, Universitat Autònoma de Barcelona, Vall d'Hebrón Hospital, Barcelona, Spain.

[6]Department of Neurosciences, Experimental Neurology, University of Leuven, Leuven, Belgium.

[7]Center for Brain & Disease Research, Laboratory of Neurobiology, Leuven, Belgium.

[8]Department of Neurology, University Hospitals Leuven, Leuven, Belgium.

[9]Stroke Division Florey Institute for Neuroscience and Mental Health, University of Melbourne, Heidelberg, Australia.

[10]Department of Neurology, Austin Health, Heidelberg, Australia [3]Faculty of Health, University of Technology Sydney, Australia.

[11]Department of Neurosciences, Experimental Neurology, KU Leuven – University of Leuven, Leuven, Belgium

[12]School of Medicine and Public Health, University of Newcastle, Newcastle, Australia.

[13]Hunter Medical Research Institute, University of Newcastle, Newcastle, Australia.

[14]Medical School, The University of Western Australia, Perth, Australia.

[15]Department of Neurology and Rehabilitation Medicine, Neurology, Skåne University Hospital, Lund, Sweden.

[16]Department of Clinical Sciences Lund, Neurology, Lund University, Lund, Sweden.

[17]IDepartment of Clinical Pathology and Genetics, Institute of Biomedicine, the Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden.

[18]Hunter Medical Research Institute, University of Newcastle, Australia.

[19]Priority Research Centre for Stroke and Traumatic Brain Injury, University of Newcastle, Australia.

[20]University of Technology Sydney, Faculty of Health, Sydney, Australia.

Corresponding author

Name: Dr Dipender Gill

Address: Department of Biostatistics and Epidemiology, School of Public Health, Medical School Building, St Mary's Hospital, Norfolk Place, Imperial College London, United Kingdom, W2 1PG

Telephone: +44 (0) 7904843810

Fax: +44 (0) 20 3313 8223

E-mail: dipender.gill@imperial.ac.uk

Cover title: Depression and outcome after ischemic stroke

Figures: 1

Subject terms: Genetic, Association Studies; Cerebrovascular Disease/Stroke

Key words: depression, functional recovery, ischemic stroke, Mendelian randomization

Category: Epidemiology/Genetics

Word count: 2,272

Abstract

Background and Purpose: Psychosocial factors can have implications for ischemic stroke risk and recovery. This study investigated the effect of genetically determined risk of depression on these outcomes using the Mendelian randomization (MR) framework.

Methods: Genetic instruments for risk of depression were identified in a discovery genome-wide association study of 246,363 cases and 561,190 controls, and further replicated in a separate population of 474,574 cases and 1,032,579 controls. Corresponding genetic association estimates for risk of ischemic stroke were taken from 60,341 cases and 454,450 controls, with those for functional outcome 3 months after ischemic stroke taken from an analysis of 6,021 patients. Following statistical power calculation, inverse variance weighted (IVW) MR was performed to pool estimates across different instruments. The Cochran's Q heterogeneity test, weighted median MR and MR-PRESSO were used to explore possible bias relating to inclusion of pleiotropic variants.

Results: There was no MR evidence for an effect of genetically determined risk of depression on ischemic stroke risk. Although suffering low statistical power, the main IVW MR analysis was suggestive of a detrimental effect of genetically determined risk of depression on functional outcome after ischemic stroke (OR of poor outcome [modified Rankin Scale≥3] per 1-standard deviation increase in genetically determined risk of depression 1.81, 95% CI 0.98-3.35, *P*=0.06). There was no evidence of heterogeneity between MR estimates produced by different instruments (Q *P*=0.26). Comparable MR estimates were obtained with weighted median MR (OR 2.57, 95%CI 1.05-6.25, *P*=0.04) and MR-PRESSO (OR 1.81, 95%CI 0.95-3.46, *P*=0.08).

Conclusions: We found no MR evidence of genetically determined risk of depression affecting ischemic stroke risk, but did find consistent MR evidence suggestive of a possible

effect on functional outcome after ischemic stroke. Given the widespread prevalence of depression related morbidity, these findings could have implications for prognostication and personalised rehabilitation after stroke.

Introduction

Functional outcome following stroke can vary considerably, ranging from complete resolution of symptoms to death. Psychosocial factors have implications for stroke recovery, and predisposition towards depression in particular can affect both physiological and psychological mechanisms[1]. Whilst observational studies can offer insight in this regard, they are often limited in inferring causality due to difficulty unravelling spurious associations related to confounding factors or reverse causation.

The Mendelian randomization (MR) technique uses genetic variants to evaluate the effect of varying an exposure on an outcome[2]. The random allocation of such variants at conception means that they are not associated with confounding factors or affected by reverse causation. While this statistical approach has been extensively used to understand the factors that cause stroke, a scarcity of genetic data had prevented its utilization for investigating functional outcome after stroke. However, the recent publication of the Genetics of Ischemic Stroke Functional Outcome (GISCOME) genome-wide association study (GWAS) has now made implementation of MR in this context feasible[3].

In this study, we perform MR analyses investigating the causal effect of genetically determined risk of depression on ischemic stroke risk and consequent functional outcome. By incorporating a range of analyses using distinct models, we present results that are robust to potential violations of the requisite assumptions.

Methods

All data supporting this work are available within the article itself and its supplementary files. These data were obtained from published studies that individually obtained appropriate ethical approval and participant consent, and no further ethical approval was required.

Genetic association estimates

Details of the studies from which genetic association estimates for MR analyses were obtained are provided in the Supplementary Methods.

Power calculations

MR power calculations were based on the available sample sizes for the respective outcomes (Supplementary Methods)[3, 4], and the genetic risk of depression instruments explaining approximately 1.2% of the variance in this exposure[5, 6].

Mendelian randomization analysis

The ratio method was used to calculate MR estimates for individual single-nucleotide polymorphisms (SNPs), using second order weights for the standard errors[2]. Fixed-effects inverse-variance weighted (IVW) meta-analysis was used to pool MR estimates across different instruments SNPs in the main analyses[2]. The Supplementary Methods details sensitivity analyses that were performed to investigate the presence of pleiotropy, where the genetic instruments affect the outcome independently of genetically determined risk of depression, to bias the MR estimate[2].

All analysis undertaken in this study used R version 3.4.1 (The R Foundation for Statistical Computing).

Results

The 56 risk of depression instrument SNPs had F-statistics ranging from 31 to 131 (Supplementary Table II), thus representing appropriate strength for MR analysis[2]. Performing power calculations for risk of ischemic stroke, we found that 80% statistical power would be achieved with a minimum detectable odds ratio (OR) of 1.11 for an mRS≥3 per standard deviation (SD) increase in genetically determined risk of depression[5]. For functional outcome after ischemic stroke, 80% statistical power would be achieved with a

minimum detectable OR of 1.85 per SD increase in genetically determined risk of depression[5].

There was no MR evidence that genetically determined risk of depression affects risk of ischemic stroke in any of the MR models considered (Figure). The main IVW MR analysis showed OR 0.98 (95%CI 0.88-1.09, *P*=0.69), although the Cochran's Q test did identify heterogeneity in the MR estimates produced by different SNPs ($P=4x10^{-3}$). Similar results were obtained in the weighted median (OR 1.00, 95%CI 0.84-1.17, *P*=0.96) and MR-PRESSO (OR 0.98, 95%CI 0.84-1.11, *P*=0.75, 2 outliers detected) sensitivity analyses. In contrast, MR evidence suggested that genetically determined risk of depression is associated with worse functional outcome after ischemic stroke. The main IVW MR analysis showed OR 1.81 (95%CI 0.98-3.35, *P*=0.06), and Cochran's Q test did not identify heterogeneity in the MR estimates produced by different SNPs (*P*=0.26). Similar results were obtained in the weighted median (OR 2.57, 95%CI 1.05-6.25, *P*=0.04) and MR-PRESSO (OR 1.81, 95%CI 0.95-3.46, *P*=0.08, no outliers detected) sensitivity analyses. Forest, radial and funnel plots depicting the ratio method MR estimates produced by individual SNPs for this analysis are provided in Supplementary Figures I-III.

Discussion

This MR study overcomes environmental confounding and reverse causation to find no evidence of an effect of genetically determined risk of depression on risk of ischemic stroke, but did find evidence suggestive of a possible effect on functional outcome after ischemic stroke. This work represents the first MR study to investigate functional outcome after stroke. The lack of association with ischemic stroke risk suggests that collider bias is unlikely to affect our analysis of functional outcome[7], and our sensitivity analyses did not reveal any evidence of pleiotropic variants biasing the MR estimates. Furthermore, by using a two-sample MR approach, we were able to maximize the data available for analyses. Despite this,

statistical power for the analysis of functional outcome was restricted, with wide 95% CIs and borderline statistical significance for the observed magnitude of effect. Use of distinct study populations for exposure and outcome genetic association estimates may also have introduced bias related to differences in the underlying populations and discrepancies in the analytical models used. Variation in the time that baseline NIHSS was measured when obtaining genetic association estimates for functional outcome may have introduced measurement error, thus also potentially reducing statistical power. Additional limitations include the use of genetic variants to evaluate risk of depression as a proxy for predisposition to depression[8]. The results should therefore not be directly extrapolated to estimate the effect of a clinical intervention for treating or preventing depression, either before or following stroke.

The lack of association of risk of depression with risk of stroke contradicts the findings from some previous observational studies (Supplementary Table IV). Indeed, observational analyses can be biased by reverse causation and confounding biases, and this may also explain the discrepancies between previous observational studies (Supplementary Table IV) and indeed the findings of our current MR analysis that largely overcomes such limitations. Depressive disorders and related tendencies are widespread and have implications for ischemic stroke patients[1]. Recovery may be affected through reduced motivation, engagement with rehabilitation and cognitive function[1]. A recent randomized controlled trial of 3,127 stroke patients did not identify any benefit of the anti-depressant fluoxetine on functional outcome at 6 months, although incidence of post-stroke depression was reduced[9]. The research question addressed in this trial differs markedly from our current study aim, which is concerned with the effect of predisposition to depression prior to ischemic stroke event on functional recovery at 3 months. Further work is warranted to replicate our findings in a larger dataset, consider distinct patient subgroups (including different ethnic groups and those

with and without recurrent stroke), and explore whether a history of depressive tendencies prior to stroke may be used to predict outcome or influence rehabilitation strategies after stroke.

Author contributions and GISCOME co-investigator contributions are detailed in the Online Supplement.

Acknowledgements

Details of all GISCOME authors and their funding sources are available in the original publication of the study's results[3]. The MEGASTROKE project received funding from sources specified at http://www.megastroke.org/acknowledgments.html. Details of all MEGASTROKE authors are available at http://www.megastroke.org/authors.html.

Sources of Funding

DG is supported by the Wellcome 4i programme at Imperial College London. NSR is in part supported by NIH-National Institute of Neurological Disorders and Stroke grants R01NS086905 and R01NS082285. RS is supported by the National Health and Medical Research Council. AL is supported by Lund University, Skåne University Hospital, the Freemasons Lodge of Instruction in Lund, the Swedish Stroke Association and Sparbanksstiftelsen Färs och Frosta. CJ is supported by the Swedish Heart and Lung Foundation and the Swedish Medical Research Council.

Conflicts of Interest and Disclosures

AL reports personal fees for Advisory Board, Speech and Seminar Participation from Bayer, Astra Zeneca, Boehringer Ingelheim, BMS Pfizer and Reneuron. All other authors declare no disclosures.

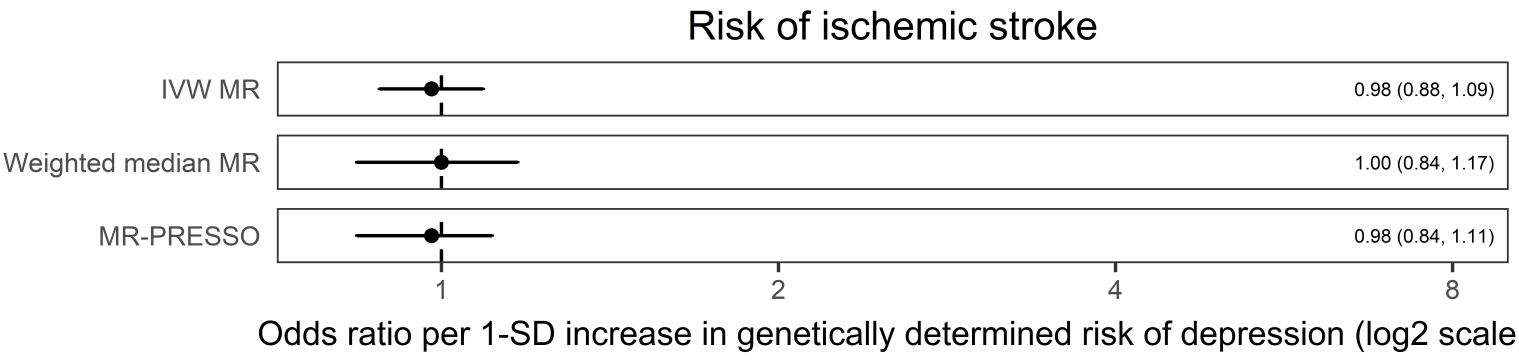
References

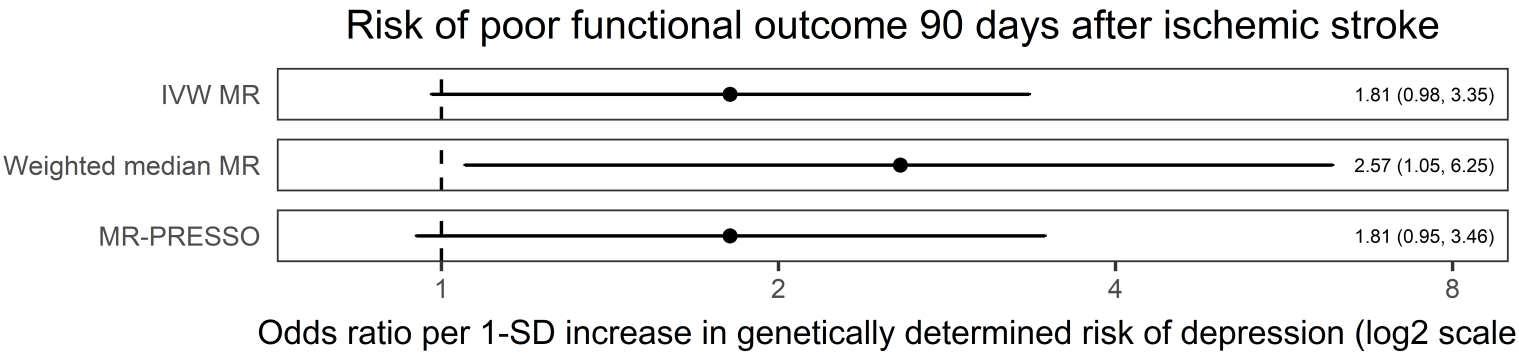
1. Terroni L, Sobreiro MFM, Conforto AB, Adda CC, Guajardo VD, de Lucia MCS, et al. Association among depression, cognitive impairment and executive dysfunction after stroke. *Dement Neuropsychol*. 2012;6:152-157
2. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol*. 2013;37:658-665
3. Soderholm M, Pedersen A, Lorentzen E, Stanne TM, Bevan S, Olsson M, et al. Genome-wide association meta-analysis of functional outcome after ischemic stroke. *Neurology*. 2019; 92:e1271-e1283
4. Malik R, Chauhan G, Traylor M, Sargurupremraj M, Okada Y, Mishra A, et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat Genet*. 2018;50:524-537
5. Brion MJ, Shakhbazov K, Visscher PM. Calculating statistical power in mendelian randomization studies. *Int J Epidemiol*. 2013;42:1497-1501
6. Howard DM, Adams MJ, Clarke TK, Hafferty JD, Gibson J, Shirali M, et al. Genome-wide meta-analysis of depression identifies 102 independent variants and highlights the importance of the prefrontal brain regions. *Nat Neurosci*. 2019;22:343-352
7. Paternoster L, Tilling K, Davey Smith G. Genetic epidemiology and mendelian randomization for informing disease therapeutics: Conceptual and methodological challenges. *PLOS Genet*. 2017;13:e1006944
8. Burgess S, Labrecque JA. Mendelian randomization with a binary exposure variable: Interpretation and presentation of causal estimates. *Eur J Epidemiol*. 2018;33:947-952

9. Dennis M, Forbes J, Graham C, Hackett M, Hankey GJ, House A, et al. Effects of fluoxetine on functional outcomes after acute stroke (FOCUS): A pragmatic, double-blind, randomised, controlled trial. *Lancet*. 2019;393:265-274

Figure Legend

Figure. Forest plot summarising the results of the MR analyses, measured in odds ratio of ischemic stroke and poor functional outcome (modified Rankin Scale ≥3) respectively per 1-standard deviation (SD) increase in genetically determined risk of depression. IVW MR: inverse-variance weighted Mendelian randomization; PRESSO: pleiotropy residual sum and outlier.


Risk of ischemic stroke
IVW MR
Weighted median MR
MR-PRESSO
0.98 (0.88, 1.09)
1.00 (0.84, 1.17)
0.98 (0.84, 1.11)
1
2
4
8
Odds ratio per 1-SD increase in genetically determined risk of depression (log2 scale)


Risk of poor functional outcome 90 days after ischemic stroke
IVW MR
Weighted median MR
MR-PRESSO
1.81 (0.98, 3.35)
2.57 (1.05, 6.25)
1.81 (0.95, 3.46)
1
2
4
8
Odds ratio per 1-SD increase in genetically determined risk of depression (log2 scale)