APOE ε4 is associated with younger age at ischemic stroke onset but not with stroke outcome

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Stroke outcome is determined by a complex interplay, where age and stroke severity are predominant predictors. Studies on hemorrhagic stroke indicate that APOE genotype is a predictor of poststroke outcomes, but results from studies on ischemic stroke are more conflicting. There is 1 study suggesting an influence of APOE genotype on age at ischemic stroke onset, and sex-specific effects on outcome have been reported. Taken together, there is a need for larger studies on APOE and ischemic stroke outcomes with integrated information on age, severity, and sex.

The 3 common APOE alleles ε2, ε3, and ε4 can be separated by a combination of 2 single nucleotide polymorphisms (SNPs), rs429358 and rs7412. Thus, associations with APOE alleles are not directly captured in a regular genome-wide association study (GWAS), where each SNP is investigated separately. We derived the 3 common APOE alleles and investigated the interplay between APOE, age at ischemic stroke onset, severity, sex, and outcome within a large international collaboration, the Genetics of Ischaemic Stroke Functional Outcome (GISCOME) network.

Methods

The design and results of the first GWAS on ischemic stroke outcome within GISCOME have been reported, and the present study comprises the 6,165 cases included in this GWAS. Each center individually obtained ethical approval and participant consent. Baseline stroke severity was assessed by the NIH Stroke Scale and 3-month functional outcome by the modified Rankin Scale (mRS). Genotyping was performed with SNP arrays with subsequent imputation to the 1000 Genomes Phase 3 reference panel as described. In the present study, we investigated effects of APOE minor alleles ε4 and ε2 separately in comparison to the most common allele ε3. To this end, ε4 allele count was defined as the continuous imputed minor allele dosage of rs429358 (C), excluding samples with minor allele dosage >0.4 for rs7412 (T), and vice versa for ε2, as depicted in figure, A. Each cohort was analyzed separately, and for each analysis, cohorts with an effective number of minor alleles ≤5 or an extreme effect size (β > 100) were excluded. Results from the remaining cohorts were meta-analyzed using inverse variance-weighted fixed effects models.

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Genetics of Ischaemic Stroke Functional Outcome (GISCOME) network coinvestigators are listed in appendix 2 at the end of the article.

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effects, unless there were signs of heterogeneity ($\text{heterogeneity} \leq 0.05$) in which case random effects were used.

We used directed acyclic graphs (DAGs) to investigate associations between $\text{APOE}$, age at stroke onset, stroke severity, and outcome. A DAG illustrates associations between variables according to a definite direction of causality as depicted by the arrows connecting the variables. For instance, $\text{APOE}$ can influence age at stroke onset and/or stroke severity, but reverse causality is unlikely as $\text{APOE}$ genotype is determined at conception. As age and stroke severity are well-established predictors of stroke outcome, we aimed to account for both possible direct effects of $\text{APOE}$ on outcome and/or indirect effects via associations with age and/or stroke severity as depicted by the 3 different arrows originating from $\text{APOE}$ in figures, B and D. All genetic analyses were adjusted for ancestry (the 5 first principal components), and adjustments for age and stroke severity were made as indicated (figure, B and D). Prespecified sex-stratified analyses were performed. Associations between allele count, age, and stroke severity were analyzed by linear regression. Associations with outcome were analyzed with logistic (dichotomized mRS score 0–2 vs 3–6) and ordinal logistic regression.

Results

Increasing allele count of $\epsilon 4$ was associated with younger age at stroke onset ($\beta = -1.8, p < 0.001$, figure, B). This association was consistent across a majority of cohorts (figure, C), significant in
both sexes and in cases with first-ever stroke only (data not shown). There was an association between ε4 allele count and favorable outcome (mRS score ≤2) when adjusting only for ancestry, but this association was no longer retained after additional adjustment for age and stroke severity (figure, B).

For ε2 allele count, we found a direct association with poor outcome (mRS score >2) in men after adjustment for ancestry, age, and stroke severity (figure, D). No such association was detected in the whole sample or in women. Neither ε4 nor ε2 allele count showed association with stroke severity.

Discussion

This is the largest meta-analysis with combined information on common APOE alleles, age at ischemic stroke onset, severity, and outcome to our knowledge. We found that increasing ε4 allele count was associated with younger age at stroke onset, which is in line with a previous meta-analysis of candidate gene studies. However, we found no evidence of a direct effect of ε4 on outcome, similar to 1 recent candidate gene study (N = 786) and 1 meta-analysis (N = 1,453).

Future studies should elucidate the biological mechanisms behind the association between APOE ε4 allele count and younger age at ischemic stroke onset. However, possible mechanisms include effects of altered lipid metabolism. In a pooled analysis, where associations between APOE genotype and several biomarkers were investigated, there was an apparent dose-response segregation of low-density lipoprotein cholesterol concentrations by APOE genotype, with the highest values in subjects homozygote for the APOE ε4 allele. Furthermore, the same ordering was observed for increasing carotid intima-media thickness and risk of ischemic stroke.

In the sex-stratified analysis, we found an association between increasing ε2 allele count and poor outcome in men. Sex-specific effects of APOE on ischemic stroke outcome have been reported and are not unreasonable to assume from a cardiovascular viewpoint. The ε2 allele has been associated with increasing white matter disease (WMD) in patients with ischemic stroke, and WMD is in turn associated with poor stroke outcome. Our results might thus be related to a higher prevalence of WMD in male ε2 carriers. However, as we lacked data on WMD for all participants, this hypothesis remains speculative.

The GISCOME study has the advantage of being the largest sample of genetic and ischemic stroke outcome data available. Study limitations have been previously discussed. In addition, the sample size for the sex-stratified analyses in our present study was small, and we used imputed values from SNP arrays to establish common APOE alleles. However, imputation based on the 1000 Genomes reference panel has been reported reliable in inferring these APOE alleles.

In conclusion, this study shows that APOE ε4 carriers have a younger age at ischemic stroke onset. We also detected worse functional outcome in male ε2 carriers, a result needing replication. Given these findings, even larger studies would be of interest to investigate associations between APOE alleles and ischemic stroke outcomes in different age and sex strata.

Study funding

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Disclosure

C. Lagging, E. Lorentzen, T. Stanne, A. Pedersen, and M. Söderholm report no disclosures. J. Cole reports receiving the AHA-Bayer Discovery Grant (Grant-171BDG33700328), K. Jood, R. Lemmens, C. Phuah, N. Rost, V. Thijs, D. Woo, and J.M. Maguire report no disclosures relevant to the manuscript. A. Lindgren reports personal fees for advisory board, speech, and seminar participation from Bayer, AstraZeneca, Boehringer Ingelheim, BMS Pfizer, and Reneuron. C. Jern reports no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

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|                    | Sahlgrenska University Hospital, Gothenburg, Sweden |                    |                                                        |
| Erik Lorentzen, MSc| University of Gothenburg, Gothenburg, Sweden   | Author             | Analysis and interpretation of the data and drafting parts of the manuscript for intellectual content |
### Appendix 1 (continued)

| Name                  | Location                                                                 | Role          | Contribution                                                                 |
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### Appendix 1 (continued)

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### Appendix 2 Coinvestigators

| Name                  | Location                                                                 | Role          | Contribution                                                                 |
|-----------------------|--------------------------------------------------------------------------|---------------|------------------------------------------------------------------------------|
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| Israel Fernandez-Cadenas, MSc, PhD | Hospital de Sant Pau, Spain; Vall d'Hebrón Hospital, Barcelona, Spain | Co-investigator | Primary investigator of one of the cohorts in GISCOME and major role in the acquisition of data |
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**Appendix 2** (continued)

| Name                        | Location                          | Role               | Contribution                                                                 |
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| Cathie Sudlow, MD, PhD      | University of Edinburgh, UK       | Co-investigator    | Primary investigator of one of the cohorts in GISCOME and major role in the acquisition of data |

**References**

1. Martinez-Gonzalez NA, Sudlow CL. Effects of apolipoprotein E genotype on outcome after ischaemic stroke, intracerebral haemorrhage and subarachnoid haemorrhage. J Neurol Neurosurg Psychiatry 2006;77:1329–1335.
2. Biffi A, Anderson CD, Jagella JM, et al. APOE genotype and extent of bleeding and outcome in lobar intracerebral haemorrhage: a genetic association study. Lancet Neurol 2011;10:702–709.
3. Lindgren A, Maguire J. Stroke recovery genetics. Stroke 2016;47:2427–2434.
4. Kumar A, Kumar P, Frasad M, Miera S, Kishor Pandit A, Chakravarty K. Association between apolipoprotein epsilon4 gene polymorphism and risk of ischemic stroke: a meta-analysis. Ann Neurosciences 2016;23:113–121.
5. Gromadzka G, Baranska-Gieruszczak M, Sarynska-Dlugosz I, Ciesielka A, Colon-Luwowska A. The APOE polymorphism and 1-year outcome in ischemic stroke: genotypetype–gender interaction. Acta Neurol Scand 2007;116:392–398.
6. Soderholm M, Pedersen A, Lorenzen E, et al. Genome-wide association meta-analysis of functional outcome after ischemic stroke. Neurology 2019;92:e1271–e1283.
7. Zhang Y, Liu S, Yue W, et al. Association of apolipoprotein E genotype with outcome in hospitalized ischemic stroke patients. Medicine (Baltimore) 2017;96:e9964.
8. Khan TA, Shah T, Prieto D, et al. Apolipoprotein E genotype, cardiovascular biomarkers and risk of stroke: systematic review and meta-analysis of 14,015 stroke cases and pooled analysis of primary biomarker data from up to 60,883 individuals. Int J Epidemiol 2013;42:475–492.
9. Lemmens R, Gomar A, Schroten M, Thijs V. Association of apolipoprotein E F0652 with white matter disease but not with microbleeds. Stroke 2007;38:1185–1188.
10. Radmanesh F, Devan WJ, Anderson CD, Rosand J, Falcone GJ. Accuracy of imputation to infer unobserved APOE epsilon alleles in genome-wide genotyping data. Eur J Hum Genet 2014;22:1239–1242.

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