

APOE $\epsilon 4$ is associated with younger age at ischemic stroke onset but not with stroke outcome

Cecilia Lagging, MD, Erik Lorentzen, MSc, Tara M. Stanne, PhD, Annie Pedersen, MD, PhD, Martin Söderholm, MD, PhD, John W. Cole, MD, MSc, Katarina Jood, MD, PhD, Robin Lemmens, MD, PhD, Chia-Ling Phuah, MD, MMSc, Natalia S. Rost, MD, MPH, Vincent Thijs, MD, PhD, Daniel Woo, MD, MSc, Jane M. Maguire, RN, PhD, Arne Lindgren, MD, PhD, and Christina Jern, MD, PhD, on behalf of the Genetics of Ischaemic Stroke Functional Outcome (GISCOME) network and the International Stroke Genetics Consortium

Correspondence

Dr. Lagging
cecilia.lagging@gu.se

Neurology® 2019;93:849-853. doi:10.1212/WNL.0000000000008459

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,^{1,2} but results from studies on ischemic stroke are more conflicting.^{1,3} There is 1 study suggesting an influence of *APOE* genotype on age at ischemic stroke onset,⁴ and sex-specific effects on outcome have been reported.⁵ 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 $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ 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,⁶ 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.⁶ In the present study, we investigated effects of *APOE* minor alleles $\epsilon 4$ and $\epsilon 2$ separately in comparison to the most common allele $\epsilon 3$. To this end, $\epsilon 4$ 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 $\epsilon 2$, 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 ($\beta > 100$) were excluded. Results from the remaining cohorts were meta-analyzed using inverse variance-weighted fixed

From the Department of Laboratory Medicine (C.L., T.M.S., A.P., C.J.), Institute of Biomedicine, the Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; Department of Clinical Genetics and Genomics (C.L., A.P., C.J.), Sahlgrenska University Hospital, Gothenburg, Sweden; Bioinformatics Core Facility (E.L.), University of Gothenburg, Sweden; Department of Clinical Sciences Lund (M.S., A.L.), Neurology, Lund University, Sweden; Department of Neurology and Rehabilitation Medicine (M.S.), Neurology, Skåne University Hospital, Malmö, Sweden; Department of Neurology (J.W.C.), Baltimore VA Medical Center and University of Maryland School of Medicine, Baltimore, MD; Department of Neurology (K.J.), Sahlgrenska University Hospital, Gothenburg, Sweden; Department of Clinical Neuroscience (K.J.), Institute of Neuroscience and Physiology, the Sahlgrenska Academy University of Gothenburg, Sweden; Neurosciences (R.L.), Experimental Neurology, KU Leuven—University of Leuven; VIB—Center for Brain & Disease Research (R.L.); Department of Neurology (R.L.), University Hospitals Leuven, Belgium; Department of Neurology (C.-L.P.), Washington University School of Medicine in St. Louis; J. Philip Kistler Stroke Research Center (N.S.R.), Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston; Stroke Division (V.T.), Florey Institute of Neuroscience and Mental Health, University of Melbourne, Heidelberg, Victoria, Australia; Department of Neurology (V.T.), Austin Health, Heidelberg, Victoria, Australia; Department of Neurology and Rehabilitation (D.W.), University of Cincinnati College of Medicine, OH; Faculty of Health (J.M.M.), University of Technology Sydney, Sydney, Australia; Hunter Medical Research Centre (J.M.M.), Newcastle, Australia; and Department of Neurology and Rehabilitation Medicine (A.L.), Neurology, Skåne University Hospital, Lund, Sweden.

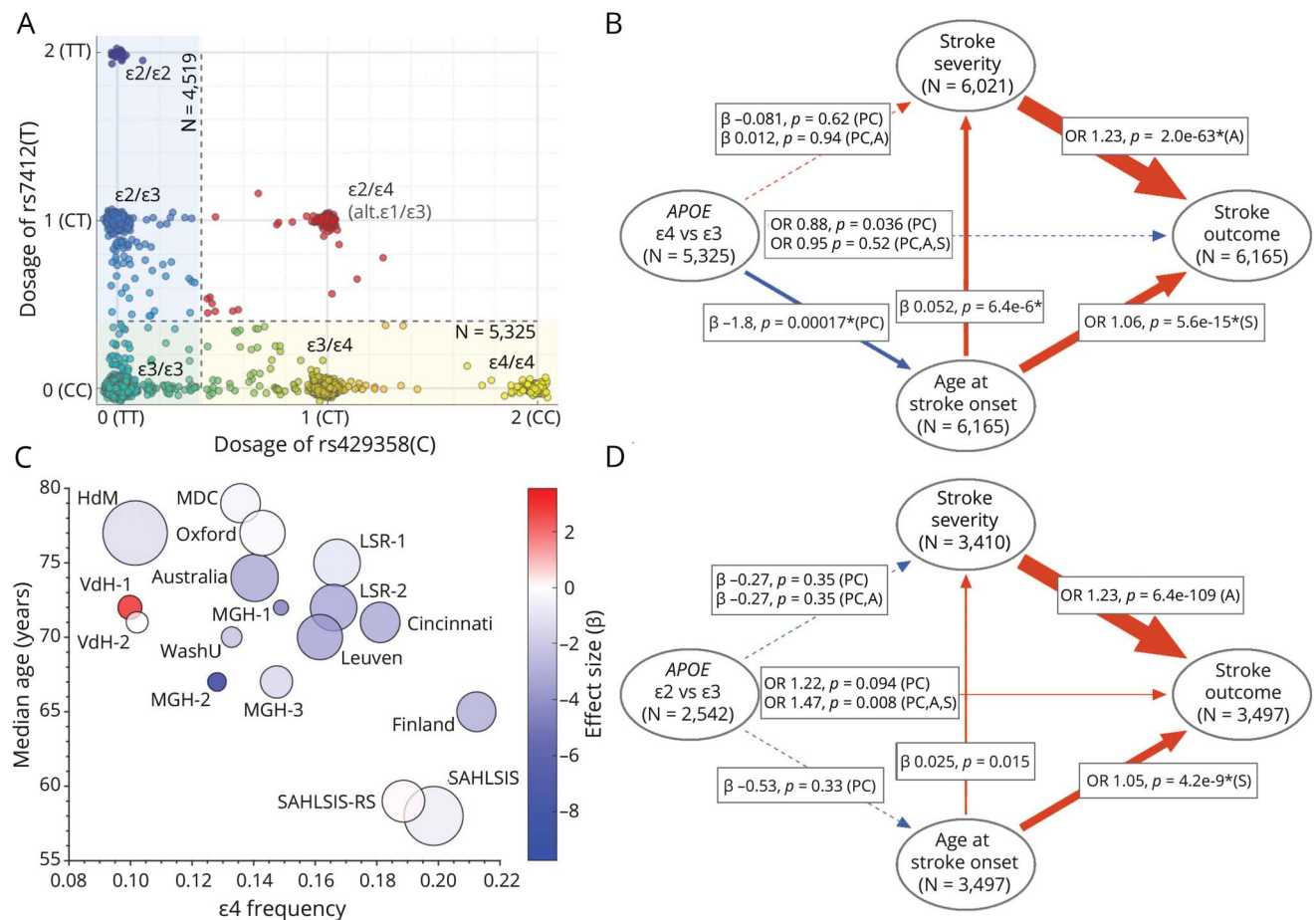
Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

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

The Article Processing Charge was funded by the Swedish Research Council.

This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (CC BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Figure *APOE* allele distribution and associations with age at ischemic stroke onset, stroke severity, and outcome



(A) Distribution of *APOE* alleles according to the SNPs rs429358 and rs7412. As the allele counts are inferred from imputation, they are given as a continuum between homozygosity for the major allele and homozygosity for the minor allele, with slightly shifted positions to improve clarity in the graph. In the analyses of ε4 vs ε3, 5,325 cases were included, and 4,519 cases were included in the analyses of ε2 vs ε3. Red positions correspond to cases excluded from both analyses. (B and D) Directed acyclic graphs (DAGs) displaying associations between *APOE* allele count and age at ischemic stroke onset, stroke severity (baseline NIH Stroke Scale score), and dichotomized 3-month mRS score (mRS score 0–2 vs 3–6). N indicates number of cases with nonmissing information, except for *APOE* allele count where N refers to maximum number of cases included in the analysis, that is, cases with allele dosage ≤ 0.4 for rs7412(T) for ε4 vs ε3 and ≤ 0.4 for rs429358(C) for ε2 vs ε3. Figure B examines associations with ε4 allele count and includes both sexes, whereas figure D displays associations with ε2 allele count in men only. Associations are reported in the squared text boxes as β and p value derived from linear regression for associations with age and stroke severity, and OR and p value derived from logistic regression for associations with poor outcome (mRS score > 2). Adjustments are indicated in the parentheses as follows: PC, adjusted for ancestry (the 5 first principal components); A, age adjusted; S, stroke severity (baseline NIH Stroke Scale) adjusted. *Refers to result from random effects meta-analysis. Arrow thickness illustrates standardized effect size after the full adjustment specified in the respective text box. Arrow color refers to the direction of the effect. A dotted arrow indicates a nonsignificant association. (C) Bubble chart showing median age at ischemic stroke onset and ε4 allele frequency for individual cohorts in GISCOME. The cohorts are described in Söderholm et al.⁶ Bubble diameter is proportional to the number of cases. Bubble color refers to the effect size (β) of ε4 on age at stroke onset derived from linear regression. GISCOME = Genetics of Ischemic Stroke Functional Outcome; mRS = modified Rankin Scale; OR = odds ratio; SNP = single nucleotide polymorphism.

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

We used directed acyclic graphs (DAGs) to investigate associations between *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, *APOE* can influence age at stroke onset and/or stroke severity, but reverse causality is unlikely as *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 *APOE* on outcome and/or indirect effects via associations with age and/or stroke severity as depicted by the 3 different arrows originating from

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 ε4 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 $\epsilon 4$ 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 $\epsilon 2$ 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 $\epsilon 4$ nor $\epsilon 2$ 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 $\epsilon 4$ allele count was associated with younger age at stroke onset, which is in line with a previous meta-analysis of candidate gene studies.⁴ However, we found no evidence of a direct effect of $\epsilon 4$ on outcome, similar to 1 recent candidate gene study (N = 786)⁷ and 1 meta-analysis (N = 1,453).¹

Future studies should elucidate the biological mechanisms behind the association between *APOE* $\epsilon 4$ 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* $\epsilon 4$ allele.⁸ Furthermore, the same ordering was observed for increasing carotid intima-media thickness and risk of ischemic stroke.⁸

In the sex-stratified analysis, we found an association between increasing $\epsilon 2$ allele count and poor outcome in men. Sex-specific effects of *APOE* on ischemic stroke outcome have been reported⁵ and are not unreasonable to assume from a cardiovascular viewpoint. The $\epsilon 2$ allele has been associated with increasing white matter disease (WMD) in patients with ischemic stroke,⁹ and WMD is in turn associated with poor stroke outcome. Our results might thus be related to a higher prevalence of WMD in male $\epsilon 2$ 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.⁶ 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.¹⁰

In conclusion, this study shows that *APOE* $\epsilon 4$ carriers have a younger age at ischemic stroke onset. We also detected worse functional outcome in male $\epsilon 2$ 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

This study was funded by the Swedish Heart and Lung Foundation (C. Jern and A. Lindgren); the Swedish Research Council (C. Jern); the Swedish Stroke Association (C. Jern and A. Lindgren); the Swedish state under the agreement between the Swedish government and the county councils, the ALF agreement (C. Jern and A. Lindgren); the Gothenburg Foundation for Neurological Research (C. Lagging); the Freemasons Lodge of Instruction EOS in Lund (A. Lindgren); the Foundation of Färs & Frosta—one of Sparbanken Skåne's ownership foundations (A. Lindgren); Lund University (A. Lindgren); Region Skåne, Skåne University Hospital (A. Lindgren); the National Health and Medical Research Council, Australia (J.M. Maguire); the National Institutes of Health (J. Cole); the US Department of Veterans Affairs (J. Cole); the American Heart Association (AHA) Cardiovascular Genome-Phenome Study (J. Cole); and the NIH-NINDS (N.S. Rost). Funding for the entire GISCOME study is listed in Söderholm et al. Genome-wide association meta-analysis of functional outcome after ischemic stroke.

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-17IBDG33700328). 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.

Publication history

Received by *Neurology* April 3, 2019. Accepted in final form August 15, 2019.

Appendix 1 Authors

| Name | Location | Role | Contribution |
|----------------------------|---|--------|---|
| Cecilia Lagging, MD | University of Gothenburg, Gothenburg, Sweden Sahlgrenska University Hospital, Gothenburg, Sweden | Author | Interpretation of the data and drafting the manuscript for intellectual content |
| Erik Lorentzen, MSc | University of Gothenburg, Gothenburg, Sweden | Author | Analysis and interpretation of the data and drafting parts of the manuscript for intellectual content |

Continued

Appendix 1 (continued)

| Name | Location | Role | Contribution |
|----------------------------------|--|--------|--|
| Tara M. Stanne, PhD | University of Gothenburg, Gothenburg, Sweden | Author | Interpretation of the data and revising the manuscript for intellectual content |
| Annie Pedersen, MD, PhD | University of Gothenburg, Gothenburg, Sweden Sahlgrenska University Hospital, Gothenburg, Sweden | Author | Interpretation of the data and revising the manuscript for intellectual content |
| Martin Söderholm, MD, PhD | Lund University, Lund, Sweden Skåne University Hospital, Malmö, Sweden | Author | Interpretation of the data and revising the manuscript for intellectual content |
| John W. Cole, MD, MSc | University of Maryland School of Medicine and Baltimore VAMC, USA | Author | Revising the manuscript for intellectual content |
| Katarina Jood, MD, PhD | University of Gothenburg, Gothenburg, Sweden Sahlgrenska University Hospital, Gothenburg, Sweden | Author | Major role in the acquisition of data and revising the manuscript for intellectual content |
| Robin Lemmens, MD, PhD | University of Leuven; VIB, Leuven; University Hospitals Leuven, Belgium | Author | Major role in the acquisition of data and revising the manuscript for intellectual content |
| Chia-Ling Phuah, MD, MMSc | Washington University School of Medicine in St. Louis, USA | Author | Revising the manuscript for intellectual content |
| Natalia S. Rost, MD, MPH | Harvard Medical School, Boston MA, USA | Author | Major role in the acquisition of data and revising the manuscript for intellectual content |
| Vincent Thijs, MD, PhD | University of Melbourne, Heidelberg; Austin Health, Heidelberg, Australia | Author | Major role in the acquisition of data and revising the manuscript for intellectual content |
| Daniel Woo, MD, MSc | University of Cincinnati College of Medicine, OH, USA | Author | Major role in the acquisition of data and revising the manuscript for intellectual content |
| Jane M. Maguire, RN, PhD | University of Technology Sydney, Sydney, Australia Hunter Medical Research Centre, Newcastle, Australia | Author | Design and conceptualization of the GISCOME study, major role in the acquisition of data, and revising the manuscript for intellectual content |

Appendix 1 (continued)

| Name | Location | Role | Contribution |
|--------------------------------|---|--------|---|
| Arne Lindgren, MD, PhD | Lund University, Lund, Sweden Skåne University Hospital, Lund, Sweden | Author | Design and conceptualization of the GISCOME study, major role in the acquisition of data, and revising the manuscript for intellectual content |
| Christina Jern, MD, PhD | University of Gothenburg, Gothenburg, Sweden Sahlgrenska University Hospital, Gothenburg, Sweden | Author | Design and conceptualization of the GISCOME and the present study, major role in the acquisition of data, analysis and interpretation of the data, and revising the manuscript for intellectual content |

Appendix 2 Coinvestigators

| Name | Location | Role | Contribution |
|---|---|-----------------|---|
| Steve Bevan, PhD | University of Lincoln, UK | Co-investigator | Major role in the acquisition of data |
| 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 |
| Graeme J. Hankey, MD | The University of Western Australia, Perth, Australia | Co-investigator | Primary investigator of one of the cohorts in GISCOME and major role in the acquisition of data |
| Jordi Jimenez-Conde, MD | Institut Hospital del Mar d'Investigació Mèdica (IMIM), Barcelona; Universitat Autònoma de Barcelona, Spain | Co-investigator | Primary investigator of one of the cohorts in GISCOME and major role in the acquisition of data |
| Jin-Moo Lee, MD, PhD | Washington University School of Medicine in St. Louis, USA | Co-investigator | Primary investigator of one of the cohorts in GISCOME and major role in the acquisition of data |
| Christopher Levi, MD | University of Technology Sydney; University of Newcastle, Australia | Co-investigator | Primary investigator of one of the cohorts in GISCOME and major role in the acquisition of data |
| Braxton D. Mitchell, PhD, MPH | University of Maryland, Baltimore; Veterans Affairs Medical Center, Baltimore, MD, USA | Co-investigator | Major role in the acquisition of data |

Appendix 2 (continued)

| Name | Location | Role | Contribution |
|--------------------------------------|---|-----------------|---|
| Bo Norrving, MD, PhD | Lund University, Lund; Skane University Hospital, Lund and Malmö, Sweden | Co-investigator | Major role in the acquisition of data |
| Kristiina Rannikmäe, MD, PhD | University of Edinburgh, UK | Co-investigator | Major role in the acquisition of data |
| Jonathan Rosand, MD, MSc | Harvard Medical School, Boston; Broad Institute of MIT and Harvard, Cambridge; Massachusetts General Hospital, Boston | Co-investigator | Primary investigator of one of the cohorts in GISCOME and major role in the acquisition of data |
| Peter M. Rothwell, MD, PhD | University of Oxford, UK | Co-investigator | Primary investigator of one of the cohorts in GISCOME and major role in the acquisition of data |
| Rodney Scott, DSc, PhD | University of Technology Sydney; University of Newcastle, Australia | Co-investigator | Primary investigator of one of the cohorts in GISCOME and major role in the acquisition of data |
| Daniel Strbian, MD, PhD | Helsinki University Hospital, Finland | Co-investigator | Major role in the acquisition of data |
| Jonathan W. Sturm, MBChB, PhD | University of Newcastle, Australia | Co-investigator | Primary investigator of one of the cohorts in GISCOME and major role in the acquisition of data |
| 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 |

Appendix 2 (continued)

| Name | Location | Role | Contribution |
|-----------------------------------|---|-----------------|---|
| Matthew Taylor, MSc, PhD | University of Cambridge, UK | Co-investigator | Major role in the acquisition of data |
| Turgut Tatlisumak, MD, PhD | University of Gothenburg, Sweden; Helsinki University Hospital, Finland | Co-investigator | Primary investigator of one of the cohorts in GISCOME and major role in the acquisition of data |
| Bradford B. Worrall, MD | University of Virginia, Charlottesville | 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, Jagiella 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, Prasad M, Misra 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, Sarzynska-Dlugosz I, Ciesielska A, Czlonkowska A. The APOE polymorphism and 1-year outcome in ischemic stroke: genotype-gender interaction. *Acta Neurol Scand* 2007;116:392–398.
6. Söderholm M, Pedersen A, Lorentzen 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:e8964.
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, Görner A, Schrooten 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.

Neurology® Online CME Program

Earn CME while reading *Neurology*. This program is available only to online *Neurology* subscribers. Read the articles marked CME, go to Neurology.org, and click on CME. This will provide all of the information necessary to get started. The American Academy of Neurology (AAN) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to sponsor continuing medical education for physicians. *Neurology* is planned and produced in accordance with the ACCME Essentials. For more information, contact AAN Member Services at 800-879-1960.

Neurology®

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

Cecilia Lagging, Erik Lorentzen, Tara M. Stanne, et al.

Neurology 2019;93:849-853 Published Online before print October 16, 2019

DOI 10.1212/WNL.00000000000008459

This information is current as of October 16, 2019

| | |
|---|---|
| Updated Information & Services | including high resolution figures, can be found at: http://n.neurology.org/content/93/19/849.full |
| References | This article cites 10 articles, 3 of which you can access for free at: http://n.neurology.org/content/93/19/849.full#ref-list-1 |
| Permissions & Licensing | Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: http://www.neurology.org/about/about_the_journal#permissions |
| Reprints | Information about ordering reprints can be found online: http://n.neurology.org/subscribers/advertise |

Neurology® is the official journal of the American Academy of Neurology. Published continuously since 1951, it is now a weekly with 48 issues per year. Copyright © 2019 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology. All rights reserved. Print ISSN: 0028-3878. Online ISSN: 1526-632X.

