Title: *APOE* ε4 is associated to younger age of ischemic stroke onset but not to stroke outcome

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Disclosures
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INTRODUCTION

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 post-stroke outcomes[1, 2], but results from studies on ischemic stroke are more conflicting[2, 3]. There is one study suggesting an influence of *APOE* genotype on age of 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 three common *APOE* alleles (ε2, ε3, ε4) can be separated by a combination of two single nucleotide polymorphism (SNPs). Thus, associations to *APOE* alleles are not directly captured in a regular genome-wide association study (GWAS), where each SNP is investigated separately. We derived *APOE* common alleles and investigated the interplay between *APOE*, age of ischemic stroke onset, severity, sex and outcome within a large international collaboration; the Genetics of Ischemic 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 appropriate ethical approval and participant consent. Baseline stroke severity was scored by the NIH Stroke Scale (NIHSS) 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]. Genotyping of the SNPs rs429358 and rs7412 can distinguish *APOE* alleles ε2, ε3 and ε4. 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 1A. 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, unless there were signs of heterogeneity ($P_{heterogeneity}$≤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 of 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 to age and/or stroke severity as depicted by the three different arrows originating from *APOE* ε4 vs ε3 and ε2 vs ε3 in the DAGs in Figure 1B and D, respectively. Thus, adjustments were made accordingly. In addition, all genetic analyses were adjusted for ancestry (the five first principal components). Prespecified sex-stratified analyses were performed. Associations between allele count, age and stroke severity were analyzed by linear regression. Associations to outcome were analyzed with logistic (dichotomized mRS 0-2 vs 3-6) and ordinal logistic regression.

RESULTS

Increasing allele count of ε4 was associated with younger age of stroke onset (β -1.8, *P*<0.001, Figure 1B). This association was consistent across a majority of cohorts (Figure 1C), 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≤2) when adjusting only for ancestry, but this association was no longer retained after additional adjustment for age and stoke severity (Figure 1B).

For ε2 allele count, we found a direct association to poor outcome (mRS>2) in men after adjustment for ancestry, age and stroke severity (Figure 1D). No such association was detected in the whole sample or in women. Neither ε4 nor ε2 allele count showed association to stroke severity.

DISCUSSION

This is the largest meta-analysis with combined information on *APOE* common alleles, age of ischemic stroke onset, severity and outcome to our knowledge. We found that increasing ε4 allele count was associated with younger age of stroke onset, a finding that is in line with a previous meta-analysis of candidate gene studies[4]. However, we found no evidence of a direct effect of ε4 on outcome, similar to one recent candidate gene study (n=786)[7] and one meta-analysis (n=1,453)[2].

Future studies will have to elucidate the mechanisms behind the association between *APOE* ε4 allele count and younger age of ischemic stroke onset. As there are well-known associations between *APOE* and lipoproteins, putative mechanisms include effects on lipids and atherosclerosis. In line with this, a large pooled analysis of studies on *APOE* genotype and lipids and other biomarkers, found a clear dose-response association between *APOE* and

low-density lipoprotein cholesterol (LDL) as well as carotid intima media thickness (IMT), with highest LDL and IMT in subjects homozygote for the *APOE* ε4 allele[8].

In the sex stratified analysis, we found an association between increasing ε2 allele count and poor outcome in men. To the best of our knowledge, this association is novel. However, sex specific effects of *APOE* on ischemic stroke outcome have previously been reported[5] and are not unreasonable to assume from a cardiovascular viewpoint. The ε2 allele has also been associated with increased bleeding volume and poor outcome following lobar intracranial hemorrhage[1]. Very speculatively, our results might thus be explained by a higher frequency of hemorrhagic transformation in male ε2 carriers.

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, for the sex-stratified analyses in this study our sample size was small. Furthermore, we used imputed values from SNP arrays to establish *APOE* alleles. However, imputed genotyping of these SNPs against the 1000 Genomics reference panel has previously been reported reliable in inferring the three common *APOE* alleles[9].

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

FIGURE 1.

Figure title: *APOE* allele distribution and associations to age of ischemic stroke onset, stroke severity and outcome.

Figure legend:

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 jittered positions in the graph to avoid over-plotting. N=5325 cases were included in the analyses of ε4 vs ε3 and N=4519 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 of ischemic stroke onset, stroke severity (baseline NIH Stroke Scale score), and dichotomized 3-month modified Rankin scale score (mRS score 0-2 vs 3-6). N indicates number of cases with non-missing information, except for *APOE* allele count where N refers to maximum number of cases included in the analysis, i.e. cases with allele dosage ≤0.4 for rs7412(T) for ε4 vs ε3, and ≤0.4 for rs429358(C) for ε2 vs ε3. Figure 1B examines associations to ε4 allele count and includes both sexes whereas figure 1D displays associations to ε2 allele count in men only. Associations are reported in the squared text boxes as β, *P* value derived from linear regression for associations to age and stroke severity, and odds ratios (OR), *P* value derived from logistic regression for associations to poor outcome (mRS>2). Adjustments are indicated in the parentheses as follows: PC, adjusted for ancestry (the five first principal components); A, age adjusted; S, stroke severity (baseline NIHSS) 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 non-significant association.

C: Bubble chart showing median age of ischemic stroke onset and ε4 allele frequency for individual cohorts in GISCOME. The cohorts are described in Söderholm M, Pedersen A, Lorentzen E, et al[6]. Bubble diameter is proportional to the number of cases. Bubble color refers to the effect size (β) of ε4 on age of stroke onset derived from linear regression.

APPENDIX 1 – list of authors

Role – ”Author” for all listed below.

Name	Location	Contribution
Cecilia Lagging, MD	University of Gothenburg, Gothenburg, Sweden Sahlgrenska University Hospital, Gothenburg, Sweden	Interpretation of the data, drafting the manuscript for intellectual content
Erik Lorentzen, MSc	University of Gothenburg, Gothenburg, Sweden	Analysis and interpretation of the data, drafting parts of the manuscript for intellectual content
Tara M Stanne, PhD	University of Gothenburg, Gothenburg, Sweden	Interpretation of the data, revising the manuscript for intellectual content
Annie Pedersen, MD	University of Gothenburg, Gothenburg, Sweden Sahlgrenska University Hospital, Gothenburg, Sweden	Interpretation of the data, revising the manuscript for intellectual content
Martin Söderholm, MD, PhD	Lund University, Lund, Sweden Skåne University Hospital, Lund and Malmö, Sweden	Interpretation of the data, revising the manuscript for intellectual content
John W Cole, MD, MS	University of Maryland School of Medicine and Baltimore VAMC, USA	Revising the manuscript for intellectual content
Katarina Jood, MD, PhD	University of Gothenburg, Gothenburg, Sweden Sahlgrenska University Hospital, Gothenburg, Sweden	Major role in the acquisition of data, revising the manuscript for intellectual content
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Arne Lindgren, MD, PhD	Lund University, Lund, Sweden Skåne University Hospital, Lund, Sweden	Design and conceptualization of the GISCOME study, major role in the acquisition of data, revising the manuscript for intellectual content
Christina Jern, MD, PhD	University of Gothenburg, Gothenburg, Sweden Sahlgrenska University Hospital, Gothenburg, Sweden	Design and conceptualization of the GISCOME and the present study, major role in the acquisition of data, analysis and interpretation of the data, revising the manuscript for intellectual content

APPENDIX 2 – list of co-investigators

Role – "Co-investigator in the Genetics of Ischemic Stroke Functional Outcome (GISCOME) network" for all listed below.

Name	Location	Contribution
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Israel Fernandez-Cadenas, MSc, PhD	Hospital de Sant Pau, Spain; Vall d'Hebrón Hospital, Barcelona, Spain	Primary investigator of individual cohort in GISCOME, major role in the acquisition of data
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Jonathan Rosand, MD, MSc	Harvard Medical School, Boston; Broad Institute of MIT and Harvard, Cambridge; Massachusetts General Hospital, Boston	Primary investigator of individual cohort in GISCOME, major role in the acquisition of data

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