Causal relationships between ischemic stroke and epilepsy: a Mendelian randomization study

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Abstract

Background

Epidemiological evidence suggests that ischemic stroke may increase the risk of epilepsy. However, the causal relationship between these two conditions is currently inconclusive. We aimed to elucidate the possible causal relationship between ischemic stroke and related subtypes of epilepsy by a two-sample Mendelian randomization (MR) analysis.

Methods

We extracted 11 single nucleotide polymorphisms (SNPs) significantly associated with ischemic stroke from a large genomic association study (GWAS) of European individuals (440328 individuals) as instrumental variables and used the GWAS of epilepsy-related subtypes provided by the International League Against Epilepsy as an outcome data source. Inverse variance weighting (IVW) was used as the primary method to assess effect estimates. Sensitivity analysis was performed using weighted median, MR-Egger and MR-PRESSO to assess heterogeneity and pleiotropy.

Results

In the IVW random-effects model, 11 SNPs were used as instrumental variables, and a significant causal relationship was found between genetic susceptibility to ischemic stroke and generalized epilepsy (OR, 1.24; 95% confidence interval (CI), 1.09, 1.42; P = 0.0009). The results of the weighted median and MR-Egger methods were in general agreement. Sensitivity analysis showed no heterogeneity or polymorphism. Reverse MR analysis failed to detect a causal relationship between generalized epilepsy and ischemic stroke. In addition, we found no causal relationship between ischemic stroke and focal epilepsy (OR, 1.06; 95% confidence interval (CI), 0.93, 1.20; P = 0.3957).

Conclusion

There is a causal relationship between ischemic stroke and the occurrence of generalized epilepsy, but not with focal epilepsy.

Introduction

Epilepsy, one of the most common neurological disorders, affects approximately 70 million people worldwide, and at a rate of 50.4 to 81.7 cases per 100,000 people per year[1; 2]. Idiopathic epilepsy is thought to have no obvious triggers, but triggered epilepsy seem to be associated with many conditions, such as cranial trauma[3], intracranial infections[4], intracranial tumors, and cerebrovascular diseases[5].
Although the pathogenesis of epilepsy is unclear, some studies suggest a preference for syndromes with multiple risk factors and strong genetic susceptibility to induce epilepsy[6], such as stroke.

Stroke is a common cardiovascular disease and is the second leading cause of death in the world [7], has been reported with approximately 10% of epilepsy and 55% of newly diagnosed epilepsy in older adults being caused by stroke[8]. What’s more, the distribution of seizures is bimodal: peak seizures are always seen in childhood and after age 60[6], which suggests that damage to the central system due to stroke may be a cause of epilepsy that cannot be ignored. However, the data on the occurrence of stroke-induced epilepsy vary considerably at this stage[9; 10] and are related to many confounding factors such as timing of diagnosis and treatment, rehabilitation care and economic conditions[11; 12; 13; 14]. Strokes are classified as ischemic strokes and hemorrhagic strokes. In a cohort study, children with ischemic stroke were found to have a 27.8-fold increased risk of future epilepsy compared to control children[15]. Therefore, establishing a causal relationship between epilepsy and ischemic stroke can help improve the understanding of the pathogenesis of the disease and provide new evidence for clinical management and prognostic assessment.

Mendelian randomization (MR) as a statistical method is to use genetic variation associated with exposure factors as instrumental variables to assess the relationship between exposure factors and outcomes which relies on the natural, random classification of genetic variation during meiosis, resulting in a random distribution of genetic variation in the population.[16; 17; 18] Thus, MR is similar to randomized controlled studies in that it avoids the effects of confounding factors and reverse causality. In the present study, we performed a comprehensive two-sample MR analysis with the aim of exploring the causal relationship between ischemic stroke and epilepsy.

**Materials And Methods**

**Data sources**

For ischemic stroke, we obtained summary statistics on stroke from the publicly available database provided by the MEGASTROKE consortium, which includes 34217 cases and 406111 controls of European ancestry. The MEGASTROKE consortium defined stroke as a rapidly developing symptom of a local (or global) disturbance of brain function with no apparent etiology other than a vascular source that lasts more than 24 hours or results in death. The study received prior approval from the relevant institutional review board and informed consent was obtained from each study participant[19].

In addition, we obtained genome-wide association study (GWAS) statistics from the International League Against Epilepsy (ILAE) that included epilepsy-related subtypes. The focal epilepsy dataset contained 9,671 cases and 29,677 controls, and the generalized epilepsy dataset contained 3,769 cases and 29,677 controls, with specific diagnostic criteria, demographic characteristics. More details about epilepsy cases available in the original study[20].
All GWAS summary statistics used in the MR analysis are shown in Table 1. All data for this study were obtained from publicly available summary statistics from GWAS and ethical approval can be found in the original study.

Data processing

First, 18 single nucleotide polymorphisms (SNPs) with significant (P < 5×10^{-8}) association with ischemic stroke were screened from the GWAS dataset of ischemic stroke and then the parameter $r^2$ threshold was set to 0.1 and kilobase pair (kb) to 10000 to exclude linkage disequilibrium. The remaining 17 SNPs were then matched with SNPs in the epilepsy database, and finally 11 SNPs significantly associated with stroke were obtained as instrumental variables for subsequent statistical processing. SNPs with a minor allele frequency of < 0.01 were also excluded to avoid potential the statistical bias from the original GWAS since they usually carry with low confidence. The $R^2$ and F values were also calculated to determine the degree of explanation of exposure by instrumental variables and the presence of weak instrumental variables, respectively. $R^2$ was calculated as $R^2 = 2 \times \text{EAF} \times (1-\text{EAF}) \times \text{Beta}^2$, and F was calculated as $F = R^2 \times (N-2)/(1- R^2)\ [21]$.

Statistical analysis

This MR study is based on three hypotheses, as described in Fig. 1.

We performed a series of Mendelian randomization analyses to investigate the causal relationship between ischemic stroke and epilepsy.

First, we regressed exposure (genetic variance in ischemic stroke) and outcome (genetic variance in epilepsy) and analyzed the causal relationship between ischemic stroke and epilepsy using the inverse variance weighted random effects (IVW) method, which is mainly used for causal inference of genes and will provide the most precise results, and therefore we used it as the primary method for MR [22]. In addition, we supplemented it with MR Egger and weighted median methods to identify any bias in assessing the MR hypothesis. The major difference between the MR-Egger method and IVW is that MR-Egger considers the presence of the intercept term in the regression, and in addition it uses the inverse of the ending variance as a weight for the fit[23]. Also we applied the MR Egger intercept test to detect unbalanced horizontal pleiotropy [23]. The weighted median method provides robust estimates even if up to 50% of the genetic variance violates the assumptions [24]. In addition, we performed MR multiplicity residuals and outliers (MR − PRESSO) tests to assess the presence of multiplicity [25], which mainly detects outliers and removes them if any, and re-calculate the MR estimates. In addition, we used leave-one-out analysis to check the robustness and consistency of the results[26]. Finally, Cochran's Q statistic was used in this study to detect heterogeneity.

All MR analyses in this study were performed using R software (version 4.2.1) and the "TwoSampleMR" software package (version 0.5.6).
Results

Identification of instrumental variables

Eleven SNPs significantly associated with ischemic stroke matched in the epilepsy database were used as instrumental variables, and these instrumental variables were calculated to explain approximately 1.87% of the variance in exposure, with F-values greater than 10, indicating that the presence of weak instrumental variables is less likely to bias the results. Details of all instrumental variables can be found in Table 2.

Mendelian randomization results

The results of the IVW model based on the ILAE dataset suggest that ischemic stroke may increase the relative risk of generalized epilepsy occurrence (OR, 1.24; 95% CI, 1.09, 1.42; P = 0.0009), but not the relative risk of focal epilepsy (OR, 1.06; 95% CI, 0.93, 1.20; P = 0.3957). The specific results are shown in Fig. 2, and the related scatter plots are shown in Fig. 3.

The Cochran’Q test showed no heterogeneity between ischemic stroke and generalized epilepsy (p = 0.4506), and the results of the polymorphism test also showed no cross-sectional polymorphism in this study (p = 0.2618). In addition, no polymorphic variants or outliers were detected using the MR-PRESSO method. The results of the sensitivity test analysis of the exclusion showed that the inclusion of SNPs had no significant effect on the results, as shown in Fig. 4.

In the reverse MR analysis from epilepsy to ischemic stroke, 11 SNPs were selected as IVs. In the IVW method, we found no relevant evidence of a causal relationship between ischemic stroke and the risk of generalized epilepsy (OR, 1.04; 95% CI, 0.97, 1.12; p = 0.2571) (Supplementary Table S2). This was also confirmed by other MR approaches as shown in Supplementary Table S2. Sensitivity analysis verifies that epilepsy is not heterogeneous for ischemic stroke (p = 0.4354). The pleiotropy test indicated no pleiotropy in the analysis (p = 0.6877), and the results were stable when excluding any single SNP. Collectively, there is currently no evidence for an inverse causal relationship between ischemic stroke and generalized epilepsy.

Discussion

In this study, we used the two - sample MR framework by applying public data from different GWAS to decipher the causality between ischemic stroke and the risk of epilepsy. We provide genetic evidence that ischemic stroke leads to an elevated risk of developing epilepsy. In addition, no heterogeneity or horizontal pleiotropy was detected. Our findings suggest the need for timely intervention after ischemic stroke to prevent epilepsy.

At one time the relationship between stroke and epilepsy was thought to be bidirectional. One study showed that a quarter of all epilepsy patients over the age of 65 experience a stroke seizure[27]. A
A retrospective cohort study published in 2015 included a total of 21,035 epilepsy cases and 16,638 controls, and the results indicated that the incidence of stroke after seizures was 2.5 times higher in adults than in controls. After adjusting for comorbidities and other factors, the risk of stroke was as high as 60% in epilepsy cases compared to controls[28]. As a subtype of stroke, ischemic stroke is one of the leading causes of death and a major cause of disability worldwide. This study is the first to use Mendelian randomization to discuss the potential causal relationship between ischemic stroke and epilepsy.

The occurrence of epilepsy after ischemic stroke involves a variety of mechanisms, firstly, the ischemia and hypoxia caused by stroke leads to sodium pump failure, Na\(^+\) inward flow causes cell depolarization, when the depolarization reaches a certain level, calcium channels are activated and Ca\(^{2+}\) enters intracellularly in large quantities thus leading to abnormal neuronal discharge[29], meanwhile, neurotransmitter dysregulation is also an important cause of seizures, and studies have shown that with ischemia and hypoxia, glutamate and other excitatory neurotransmitters increase, which increases membrane excitability[30], and in addition, GABA inhibitory neurotransmitters undergo degeneration, weakening the inhibitory effect and lowering the membrane excitation threshold [30], which together cause abnormal neuronal firing leading to epilepsy. Stroke-induced disruption of the blood-brain barrier causes alterations in astrocyte gene expression, resulting in a decrease in the ability of astrocytes to take up K\(^+\). Elevated extracellular K\(^+\) concentrations can cause excessive neuronal firing, leading to seizures [31].

Several advantages exist in the present study. First, this study primarily used a large sample of genome-wide association studies (GWAS), which allows for a better overall analysis of stroke events. Second, in the experimental design of MR, because SNPs are randomly assigned, bias due to reverse causality or confounding factors is greatly reduced, which is not available in observational studies. Finally, we also performed several sensitivity analyses to test the consistency of the results and to correct for possible pleiotropy.

However, there are some limitations to this study. First, the epilepsy dataset provided by the ILAE used in this study had approximately 14% of non-European descent. Therefore, population stratification is likely to introduce biases here. Second, the results of the study were limited to Europeans and could not be generalized to other populations. Finally, because ischemic stroke and epilepsy are heterogeneous disorders with different genetic factors and may be differentially associated with each other, larger subgroup MR studies may be needed in the future to further explore causal relationships.

**Conclusions**

In conclusion, this MR study provides genetic evidence that ischemic stroke is associated with an increased risk of generalized epilepsy, but not focal epilepsy, in a European population. These findings may provide strong evidence for the future prevention and treatment of post-stroke epilepsy.
Declarations

Acknowledgments

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Authors’ contributions

SC designed the study. WH and XJ analyzed the data and prepared the original draft. TH and MZ revised and edited the paper. HX supervised this work. All authors have read and agreed to the published version of the manuscript.

Funding

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are publicly available in the IEU Open GWAS Project repository(http://gwas.mrcieu.au.uk).

Ethics approval and consent to participate

Not applicable. Ethical approval and informed consent for studies included in the analyses was provided in the original publications.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References


Tables

Table 1 | Details of studies and datasets used for analyses.

<table>
<thead>
<tr>
<th>Data Source</th>
<th>Phenotype</th>
<th>Sample Size</th>
<th>Cases</th>
<th>Population</th>
<th>References</th>
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</thead>
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<tr>
<td>The MEGASTROKE consortium</td>
<td>ischemic stroke</td>
<td>440328</td>
<td>34217</td>
<td>European</td>
<td>Malik R et al.</td>
</tr>
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<td>The International League Against Epilepsy Consortium</td>
<td>focal epilepsy</td>
<td>39348</td>
<td>9671</td>
<td>86% European</td>
<td>Abou-Khalil B et al.</td>
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<td></td>
<td>generalized epilepsy</td>
<td>33446</td>
<td>3769</td>
<td></td>
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</table>

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Table 2 | Association strength of SNP allele frequencies and effect alleles with ischemic stroke and epilepsy

<table>
<thead>
<tr>
<th>SNP</th>
<th>EA</th>
<th>OA</th>
<th>EAF</th>
<th>R(^2)(%)</th>
<th>F</th>
<th>Beta</th>
<th>SE</th>
<th>P-value</th>
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</thead>
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<tr>
<td>rs12445022</td>
<td>A</td>
<td>G</td>
<td>0.31</td>
<td>0.16</td>
<td>694.33</td>
<td>0.061</td>
<td>0.010</td>
<td>1.28 ×10(^{-10})</td>
</tr>
<tr>
<td>rs17035646</td>
<td>A</td>
<td>G</td>
<td>0.41</td>
<td>0.14</td>
<td>610.53</td>
<td>0.054</td>
<td>0.009</td>
<td>1.34 ×10(^{-09})</td>
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<tr>
<td>rs2107595</td>
<td>A</td>
<td>G</td>
<td>0.23</td>
<td>0.20</td>
<td>890.35</td>
<td>0.076</td>
<td>0.010</td>
<td>9.25 ×10(^{-14})</td>
</tr>
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<td>rs3184504</td>
<td>C</td>
<td>T</td>
<td>0.55</td>
<td>0.28</td>
<td>1233.78</td>
<td>-0.075</td>
<td>0.010</td>
<td>2.17 ×10(^{-14})</td>
</tr>
<tr>
<td>rs35436</td>
<td>T</td>
<td>C</td>
<td>0.38</td>
<td>0.12</td>
<td>509.69</td>
<td>-0.050</td>
<td>0.009</td>
<td>3.21 ×10(^{-08})</td>
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<tr>
<td>rs42039</td>
<td>T</td>
<td>C</td>
<td>0.23</td>
<td>0.15</td>
<td>667.45</td>
<td>-0.066</td>
<td>0.011</td>
<td>6.55 ×10(^{-09})</td>
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<tr>
<td>rs4932370</td>
<td>A</td>
<td>G</td>
<td>0.33</td>
<td>0.12</td>
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<td>0.052</td>
<td>0.009</td>
<td>2.88 ×10(^{-08})</td>
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<tr>
<td>rs4959130</td>
<td>A</td>
<td>G</td>
<td>0.14</td>
<td>0.16</td>
<td>722.82</td>
<td>0.083</td>
<td>0.014</td>
<td>2.83 ×10(^{-09})</td>
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<tr>
<td>rs6825454</td>
<td>C</td>
<td>T</td>
<td>0.31</td>
<td>0.14</td>
<td>597.66</td>
<td>0.056</td>
<td>0.009</td>
<td>7.43 ×10(^{-10})</td>
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<tr>
<td>rs6847935</td>
<td>T</td>
<td>A</td>
<td>0.33</td>
<td>0.27</td>
<td>1192.02</td>
<td>0.078</td>
<td>0.010</td>
<td>3.50 ×10(^{-16})</td>
</tr>
<tr>
<td>rs9526212</td>
<td>G</td>
<td>A</td>
<td>0.76</td>
<td>0.14</td>
<td>605.95</td>
<td>0.062</td>
<td>0.010</td>
<td>9.19 ×10(^{-10})</td>
</tr>
</tbody>
</table>

SNP, single-nucleotide polymorphism; EA, effect allele; EAF, effect allele frequency; \(R^2\), percentage of the variation explained by the SNP; F, F statistic; Beta, the per-allele effect on cannabis use; SE, standard error of Beta; P-value is for the genetic association.

Figures
Figure 1

Schematic diagram showing the assumptions of Mendelian randomization analysis of ischemic stroke and epilepsy.

<table>
<thead>
<tr>
<th>outcome</th>
<th>methods</th>
<th>OR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal epilepsy</td>
<td>IVW</td>
<td>1.06(0.93~1.20)</td>
<td>0.40</td>
</tr>
<tr>
<td>Focal epilepsy</td>
<td>WME</td>
<td>1.08(0.96~1.22)</td>
<td>0.21</td>
</tr>
<tr>
<td>Focal epilepsy</td>
<td>MR-Egger</td>
<td>1.75(0.83~3.66)</td>
<td>0.17</td>
</tr>
<tr>
<td>Focal epilepsy</td>
<td>MR-PRESSO</td>
<td>1.06(0.93~1.20)</td>
<td>0.42</td>
</tr>
<tr>
<td>Generalized epilepsy</td>
<td>IVW</td>
<td>1.25(1.09~1.42)</td>
<td>0.0009</td>
</tr>
<tr>
<td>Generalized epilepsy</td>
<td>WME</td>
<td>1.30(1.09~1.54)</td>
<td>0.0037</td>
</tr>
<tr>
<td>Generalized epilepsy</td>
<td>MR-Egger</td>
<td>1.20(0.91~4.37)</td>
<td>0.12</td>
</tr>
<tr>
<td>Generalized epilepsy</td>
<td>MR-PRESSO</td>
<td>1.25(1.09~1.42)</td>
<td>0.0077</td>
</tr>
</tbody>
</table>
Figure 2

Forest plot of MR estimates for the causal effect of ischemic stroke and epilepsy subtypes. IVW indicates inverse-variance weighted; WME, weighted median; MR-Egger, Mendelian randomization-Egger; and MR-PRESSO, Mendelian Randomization Pleiotropy Residual Sum and Outlier.

Figure 3

The Scatter plot of the MR analysis showed the relationship between ischemic stroke and epilepsy risk. (A) Genetic association of ischemic stroke-related SNPs and focal epilepsy. (B) Genetic association of ischemic stroke-related SNPs and generalized epilepsy.

Figure 4

MR leave-one-out sensitivity analysis for ischemic stroke on epilepsy. (A) Sensitivity analysis of ischemic stroke-related SNPs and focal epilepsy. (B) Sensitivity analysis of ischemic stroke-related SNPs and generalized epilepsy. The bars indicate the CI. MR indicates Mendelian randomization.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTableS1.xlsx
- SupplementaryTableS2.xlsx