Genetic Prediction of Antiglycemic Drug Targets and Risk of Epilepsy: A Mendelian Randomization Study

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Abstract

Diabetes has been linked to an increased risk of epilepsy in observational studies. The antiglycemic drugs have been shown in animal studies to improve seizures. However, whether the associations between antiglycemic drugs and epilepsy in human is not known. In this study, we conducted a Mendelian randomization investigation to assess the potential causal role of antiglycemic drug targets in epilepsy. We used the International League Against Epilepsy Data as the discovery set and FinnGen Data as the replication set. Three antidiabetic drug target genes, including ETFDH, CYP21A2, and CYP2D6 were discovered to be involved in epilepsy. ETFDH predicted as a target gene in the discovery set (IVW, OR = 1.018, 95% CI, 1.004–1.033, p = 0.009), replication set (IVW, OR = 1.074, 95% CI, 1.034–1.114, p = 0.00016) and CYP21A2 gene in the discovery set (IVW, OR = 1.029, 95% CI, 1.005–1.053, p = 0.016) and replication set (IVW, OR = 1.057, 95% CI, 1.001–1.116, p = 0.045) showed a causal association with an increased risk of epilepsy. In contrast, the CYP2D6 gene was found to be a protective factor for epilepsy in both the discovery set (IVW, OR = 0.0984, 95% CI, 0.969–0.998, p = 0.025) and the replication set (IVW, OR = 0.977, 95% CI, 0.955–1.000, p = 0.046). By searching the pharmacological effects of anti-glucose drug target gene related drugs and binding drugs in DrguBank, Metformin was found to be ETFDH gene inhibitor, showing a potential therapeutic effect on epilepsy.

Introduction

Epilepsy, which affects more than 70 million people worldwide, is a complex condition with multiple risk factors and a strong genetic predisposition, rather than a single clinical presentation and cause. Drug therapy, surgery, nerve stimulation, and diet therapy are the most common treatments for epilepsy. Most patients with epilepsy choose antiepileptic drug treatment, with the goal of controlling seizures on the assumption that the side effects of the drugs do not interfere with normal life. However, one-third of epilepsy patients do not achieve complete seizure control. Long-term seizures cause cognitive impairment, anxiety, depression, and other epilepsy-related complications. Since developing new drugs is time-consuming and expensive, reusing old drugs to treat common and rare diseases has gradually become a new trend known as drug reuse. Drug reuse, also known as drug repositioning, refers to the discovery of new uses for drugs that are not within the original clinical indications.

Diabetes has been linked to an increased risk of epileptic seizures in epidemiologic studies. Some antiglycemic drugs, such as liraglutide, sitagliptin, rosiglitazone, and metformin, have been shown in animal studies to be effective in reducing seizures and improving cognitive impairment. Randomized controlled clinical trials are the gold standard for determining drug efficacy. There is a lack of high-quality, large-scale randomized controlled trials to find studies on the effect of antiglycemic drugs on seizures in the population due to ethical reasons, long follow-up time, high cost, and other factors. Therefore, Mendelian randomization (MR) was developed as a new research method. It estimates the causal relationship between exposure and outcome using genetic variants as instrumental variables. "Exposure" in this context refers to any factor of interest that may influence the outcome, such
as modifiable lifestyle and biomarkers. Of course, it can be used to assess the causal relationship between drug target genes and diseases. Mendelian randomization maximization is thought to be superior to observational studies because it avoids endogenous problems in regression analysis, such as reverse causality, confounding factors, and measurement error.

Mendelian randomization analysis is currently being used to investigate the relationship between antihypertensive drugs and psychiatric disorders, drug targets for the treatment of Parkinson's disease, and the risk of lipid-lowering drugs, antiglycemic drugs, and Alzheimer's disease. In this study, to explore the effects of different antiglycemic drug treatments on epilepsy, we used the public GTEx-V8 database to confirm the proxy antiglycemic drug target genes of instrumental variables and performed two-sample Mendelian randomization analysis.

**Material And Methods**

**Study design.** We performed a two-sample Mendelian randomization study on 96 gene targets of 74 diabetes medications, such as metformin, glyburide, gliclazide, acarbose, miglitol, pioglitazone, repaglinide, sitagliptin, dapagliflozin, and others. The data from the International League Against Epilepsy were used in the discovery set, and the data from the FinnGen consortium were used in the replication set. As shown in Fig. 1, the intersection was used to determine the relationship between antiglycemic drugs and epilepsy to discover a new pathway for epilepsy treatment. This study used existing summary GWAS data, so separate ethical approval was not required (all prior studies had ethical approval in accordance with the Declaration of Helsinki).

Data Sources and Instrumental Variable Selection. We searched the anti-glucose drug targets in DrugBank database (http://www.drugbank.ca/) and found that 74 drugs or compounds that have been used in clinical practice and have not yet been used in clinical trials and are under investigation for the treatment of diabetes. The database yielded a total of 96 target genes for all antiglycemic drugs. And the GTEx-V8 database (https://gtexportal.org/home/), which studied the tissue specificity of gene expression and regulation using nearly 1000 people in 54 lesion tissue samples, was used to search SNPs associated with drug target genes. The brain tissues included were Brain_Amygdala, Brain_Anterior_cingulate_cortex, Brain_Caudate_basal_ganglia, Brain_Cerebellar_Hemisphere, Brain_Cerebellum, Brain_Cortex, Brain_Frontal_Cortex, Brain_Hippocampus, Brain_Hypothalamus, Brain_Nucleus_accumbens_basal_ganglia, Brain_Putam en_basal_ganglia, Brain_Spinal_cord_cervical, and Brain_Substantia_nigra.

Each SNP used as an instrumental variable met the following criteria: (1) it was strongly associated with antiglycemic drug target genes ($p < 5 \times 10^{-8}$); (2) it had no association with epilepsy; (3) it had no association with confounding factors. The selected instrumental variables were de-linkage disequilibrium (clump = 500kb $r^2 = 0.3$). Supplementary Table 1 lists the names of the 74 drugs and the 96 drug target genes.
The International League Against Epilepsy (ILAE) provided data for the discovery set from a large genome-wide association study of epilepsy. (15212 cases and 29677 controls). The original study contains a detailed description of cohort and phenotype definition, study design, genotyping quality control, and imputation, Statistical analyses. Furthermore, for our replication set, we used data from the FinnGen consortium (6260 cases and 176,107 controls), which is publicly available with FinnGen Data Freeze 6 and includes 260,405 participants. There are 16962023 variants and 2861 endpoints.

Statistical Methods. Two-sample Mendelian randomization data were analysed using the R package TwoSample Mendelian Randomization, version 0.5.6. We used the inverse variance-weighting (IVW) method for the main analysis, and since the number of SNPs identified for each drug was relatively small, we added a Wald ratio analysis to estimate the causal effect of anti-glucose drug targets on epilepsy as a single working variable to the main analysis. Furthermore, a variety of analysis methods, such as weighted median, weighted mode, and MR-Egger, are used to strengthen the causal inference. In the sensitivity analysis, the Cochran’s Q test was used to test for heterogeneity, the intercept term of the MR Egger method and the R package "MR-PRESSO" were used to test for multiple validity, and Leave-one-out sensitivity analysis was performed. The statistical validity of MR was determined using the mRND website (https://shiny.cnsgenomics.com/mRnd/), where the F-statistic represents the strength of the instrumental variable and can be calculated using the formula: $F = R^2(N - 2)(1 - R^2)$.

Results

International League Against Epilepsy: The relationship between antiglycemic drug targets and epilepsy. We discovered that 18 antiglycemic drug targets, CYP2E1, CFTR, GAA, CYP2D6, MGAM, CYP17A1, ETFDH, NFKB2, CYP21A2, FBP1, CYP3A5, HTR2A, SLC5A2, ABCC8, IGF1R, KCNJ11, LPL, and PPA RG, which are causally associated with epilepsy during the preliminary analysis phase. Figure 2 depicts the results, while Supplementary Table 2 displays the detailed results. Supplementary Figure 1–7 show scatter plots, funnel plots, forest plots, and analysis plots of one-by-one exclusion tests for those SNPS ≥ 5.

Further exploration of the relationship between antiglycemic drugs and epilepsy in the FinnGen consortium. Three antiglycemic drug targets were validated in the FinnGen consortium data and were consistent with the preliminary analysis results: ETFDH, CYP21A2, and CYP2D6 (Table 1, Figure 3). ETFDH (expression in the cerebellar hemispheres) predicted by the gene in the discovery set (IVW, OR = 1.018, 95% CI = [1.004–1.033], $p = 0.009$, Figure 4). In the replication set, ETFDH expressed in the Cortex (IVW, OR = 1.074, 95% CI = [1.034–1.114], $p = 0.00016$, Figure 5). Meanwhile, CYP21A2 (expression in the Cerebellum) predicted by gene in the discovery set (IVW, OR = 1.029, 95% CI = [1.005–1.053], $p = 0.016$, Supplementary Figure 8). In the replication set, CYP21A2 expressed in nucleus_accumbens_basal_ganglia (IVW, OR = 1.057, 95% CI = [1.001–1.116], $p = 0.045$, Supplementary Figure 9). They revealed a causal relationship between an increased risk of epilepsy. The CYP2D6, on the
other hand, may be a protective factor for epilepsy, expressing in the Anterior_cingulate_cortex in the discovery set (IVW, OR = 0.0984, 95% CI = [0.969–0.998], p = 0.025, Supplementary Figure 10) and expressing in the Cortex in the replication set (IVW, OR = 0.977, 95% CI = [0.955–1.000], p = 0.025, p = 0.046 in Supplementary Figure 11). All three antiglycemic targets had strong instrumentation (F-statistic values above the common threshold of 10). Also, there was almost no heterogeneity among the three targets in the heterogeneity test, and the sensitivity analysis showed that the causal effect was also stable (Figure 3).

**Discussion**

Mendelian randomization analysis was used for the first time to infer the effect of antiglycemic drugs on epilepsy. We discovered that the antiglucose drug target genes ETFDH, CYP21A2, and CYP2D6 are linked to epilepsy. Metformin was discovered to be a drug related to ETFDH by searching DrugBank for drugs related to the target genes of anti-glucose drugs and the pharmacological effects of binding drugs (see Table 1). The ETFDH gene increases the risk of epilepsy, and as an inhibitor of the ETFDH gene, metformin has a potential therapeutic effect on epilepsy. Metformin has been shown to slow the progression of Lafora disease (LD) in a small randomized controlled clinical trial investigating the role of metformin in epilepsy. It is well known that LD is a progressive myoclonic epilepsy for which there is no specific treatment. Our findings support the potential therapeutic effect of metformin therapy in the LD. The mechanism by which metformin improves epilepsy is unknown, but a growing number of animal studies have shown that metformin can improve seizures in a variety of ways. In a mouse model of kainic acid (KA) epilepsy, somayeh et al. found that metformin increased IL-10 secretion and inhibited IL-1β and astrocyte regeneration to achieve anti-inflammatory effects. Soraya et al. found that metformin activated AMP-activated protein kinase (AMPK) signaling pathway and decreased mammalian target of rapamycin (mTOR) expression in a pilocarpine epilepsy rat model. Jing et al. revealed that C/EBP homologous protein (CHOP) pathway expression and apoptosis induced by Status epilepticus (SE) in rats were reduced with metformin. Abdelaziz’s experimental result show that the antiepileptic of metformin in PTZ-induced epilepsy may be attributed to the impairment of oxidative stress and α-synuclein expression, as well as the upregulation of Hsp70. Although metformin has been shown to have therapeutic effects in various animal models of epilepsy, few randomized controlled trials have been conducted to detect the potential effects of metformin in the treatment of epilepsy clinically. Our findings support the clinical use of metformin for the treatment of epilepsy. Metformin has been shown in animal studies to improve cognitive impairment in epileptic mice, raising the possibility that metformin could be a promising candidate for the treatment of human epilepsy. However, more research is needed to determine its efficacy and safety.

Our study, of course, has limitations. First, we used data from European populations to avoid racial confusion, and the experimental results need to be confirmed in other ethnic populations. Second, the Mendelian randomization analysis of genetic variation as an instrumental variable more accurately reflects the long-term effect of antiglycemic drug target genes on epilepsy, whereas the effect of short-
term drug treatment on epilepsy cannot be inferred. Third, due to data availability constraints, this study was unable to conduct subgroup analysis, such as sex and age.

Our findings indicate that the antiglycemic drug target gene ETFDH can increase the risk of epilepsy, and metformin is an inhibitor of the ETFDH gene, which explains the potential therapeutic significance of metformin on epilepsy and lays the groundwork for further mechanistic research.

**Declarations**

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions: K.Z. and S.W. contributed to the conception and design of the study. K.Z., S.W., Z.X. and H.Y. collected and analysed the data. K.Z., S.W. drafted the manuscript. W.W. and Z.Q. revised the manuscript. All authors revised and approved the final version of the manuscript.

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Supplementary Material: See Figures S1-S11 and Table S1-S2 in the Supplementary Material for comprehensive analysis.

Data Availability Statement: Data from DrugBank database (http://www.drugbank.ca/) and FinnGen consortium (http://r6.finngen.fi/) are publicly available. The corresponding author has full access to all data and material and can provide availability if needed.

**References**


**Table 1**

**Table 1** MR Analysis of anti-glucose drug target genes and epilepsy. Stage refers to our MR Research stage, which is divided into discovery set and validation set. method refers to the meta method we used; Nsnp refers to the number of instrumental variables used; Beta refers to the effect size in the MR Analysis; and P-val refers to the statistical significance of the MR Analysis. Q refers to the significance of the heterogeneity test and Ple refers to the significance level of the pleiotropy test in the MR Analysis. R2 refers to the degree to which the instrumental variable explains the exposure. F is the F-test statistic. Drugs and Pharmacological action refer to the drugs related to the target genes of anti-glucose drugs and the pharmacological effects between them.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Target gene</th>
<th>Method</th>
<th>Nsnp</th>
<th>Beta</th>
<th>Pval</th>
<th>Q</th>
<th>Ple</th>
<th>R2</th>
<th>F</th>
<th>Drugs and Pharmacological action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery</td>
<td>ETFDH</td>
<td>Inverse variance weighted (fixed effects)</td>
<td>7</td>
<td>0.019</td>
<td>0.0091</td>
<td>0.24</td>
<td>0.51</td>
<td>0.68</td>
<td>19.10</td>
<td>Metformin (Yes inhibitor)</td>
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<tr>
<td>Replication</td>
<td>ETFDH</td>
<td>Inverse variance weighted (fixed effects)</td>
<td>5</td>
<td>0.130</td>
<td>0.0001</td>
<td>0.55</td>
<td>0.33</td>
<td>0.23</td>
<td>12.20</td>
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</tr>
<tr>
<td>Replication</td>
<td>ETFDH</td>
<td>Weighted median</td>
<td>5</td>
<td>0.095</td>
<td>0.0560</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Levoketoconazole (No)</td>
</tr>
<tr>
<td>Discovery</td>
<td>CYP21A2</td>
<td>Inverse variance weighted (fixed effects)</td>
<td>2</td>
<td>0.029</td>
<td>0.0166</td>
<td>NA</td>
<td>NA</td>
<td>0.19</td>
<td>20.17</td>
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<tr>
<td>Replication</td>
<td>CYP21A2</td>
<td>Inverse variance weighted (fixed effects)</td>
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<td>0.055</td>
<td>0.0451</td>
<td>0.07</td>
<td>0.24</td>
<td>0.24</td>
<td>25.92</td>
<td>Levoketoconazole (No)</td>
</tr>
<tr>
<td>Discovery</td>
<td>CYP2D6</td>
<td>Inverse variance weighted (fixed effects)</td>
<td>4</td>
<td>-0.017</td>
<td>0.0253</td>
<td>0.78</td>
<td>0.56</td>
<td>0.54</td>
<td>27.60</td>
<td>Dapagliflozin (No)</td>
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<tr>
<td>Replication</td>
<td>CYP2D6</td>
<td>Inverse variance weighted (fixed effects)</td>
<td>4</td>
<td>-0.019</td>
<td>0.0324</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Phenformin (Unkonw)</td>
</tr>
<tr>
<td>Replication</td>
<td>CYP2D6</td>
<td>Weighted median</td>
<td>13</td>
<td>-0.023</td>
<td>0.0460</td>
<td>0.69</td>
<td>0.78</td>
<td>0.90</td>
<td>12.56</td>
<td>Rosiglitazone (Unkonw)</td>
</tr>
</tbody>
</table>

**Figures**
Figure 1. Schematic representation of this study.

Schematic representation of this study.
Figure 2. The odds ratios for genetically predicted antiglycemic drug targets associated with epilepsy in the International League Against Epilepsy.

### Figure 2

The odds ratios for genetically predicted antiglycemic drug targets associated with epilepsy in the International League Against Epilepsy.
**Figure 3**

Associations of genetic proxies for antiglycemic drug targets in the International League Against Epilepsy and FinnGen. Blue, the International League Against Epilepsy, Green, the FinnGen.

<table>
<thead>
<tr>
<th>Drug target</th>
<th>Tissue-Brain</th>
<th>OR</th>
<th>[95% CI]</th>
<th>P</th>
</tr>
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<tr>
<td>CYP2D6</td>
<td>Cortex</td>
<td>0.977</td>
<td>(0.955–1.000)</td>
<td>0.046</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Anterior_cingulate_cortex</td>
<td>0.0984</td>
<td>(0.969–0.998)</td>
<td>0.025</td>
</tr>
<tr>
<td>CYP21A2</td>
<td>Nucleus_accumbens_basal_ganglia</td>
<td>1.057</td>
<td>(1.001–1.116)</td>
<td>0.045</td>
</tr>
<tr>
<td>CYP21A2</td>
<td>Cerebellum</td>
<td>1.029</td>
<td>(1.005–1.053)</td>
<td>0.016</td>
</tr>
<tr>
<td>ETFDH</td>
<td>Cortex</td>
<td>1.074</td>
<td>(1.034–1.114)</td>
<td>0.00016</td>
</tr>
<tr>
<td>ETFDH</td>
<td>Cerebellar_Hemisphere</td>
<td>1.018</td>
<td>(1.004–1.033)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

**Figure 3.** Associations of genetic proxies for antiglycemic drug targets in the International League Against Epilepsy and FinnGen. Blue, the International League Against Epilepsy, Green, the FinnGen.
Figure 4. Mendelian randomization assessment of the ETFDH expression in brain_cerebellar_hemisphere and epilepsy risk in ILAE. (A) Forest plot. Each horizontal solid line reflects the result estimated for a single snp using the Wald ratio method. The bottom red line, which reflects the risk relationship between ETFDH and epilepsy under the IVW approach. (B) Leave-one-out analysis of genetically proxies ETFDH on epilepsy risk. (C) Funnel plot. Vertical lines show causal estimates using each of the two different methods to combine all SNPS into a single instrument. (D) Scatter plot. The slope of the straight line correspond with the causal estimates using each of the five different methods.

Mendelian randomization assessment of the ETFDH expression in brain_cerebellar_hemisphere and epilepsy risk in ILAE. A.Forest plot. Each horizontal solid line reflects the result estimated for a single snp using the Wald ratio method. The bottom red line, which reflects the risk relationship between ETFDH and epilepsy under the IVW approach. B.Leave-one-out analysis of genetically proxies ETFDH on epilepsy risk. C.Funnel plot. Vertical lines show causal estimates using each of the two different methods to combine
all SNPS into a single instrument. D. Scatter plot. The slope of the straight line correspond with the causal estimates using each of the five different methods.

Figure 5. Mendelian randomization assessment of the ETFDH expression in brain_cortex and epilepsy risk in FinnGen. (See legend on previous page.)

Figure 5

Mendelian randomization assessment of the ETFDH expression in brain_cortex and epilepsy risk in FinnGen. (See legend on previous page.)
Figure 6. Mechanisms by which metformin improves seizures in animal models.

Figure 6

Mechanisms by which metformin improves seizures in animal models.

Supplementary Files

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- Supplementfiguresandtables.pdf