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Identification of the *ZEB2* gene as a potential target for epilepsy therapy and the association between rs10496964 and *ZEB2* expression

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Abstract

Objective: An association between the rs10496964 polymorphism and the *ZEB2* gene has not yet been reported, and the role of *ZEB2* in epilepsy therapy is also unclear. The aims of this research were to evaluate the role of *ZEB2* in the therapy of epilepsy and to explore the association between rs10496964 and *ZEB2* expression.

Methods: We used the expression quantitative trait loci (eQTL) dataset resource from the Brain eQTL Almanac to evaluate the association between rs10496964 and *ZEB2* expression in human brain tissue. Pathway and process enrichment analysis, protein–protein interaction analysis, and PhosphoSitePlus® analysis were then performed to further evaluate the role of *ZEB2* in the therapy of epilepsy.

Results: The rs10496964 polymorphism was found to regulate the expression of *ZEB2* in human brain tissue. The ZEB2 protein interacts with the targets of approved antiepileptic drugs, and a post-translational acetylation modification of ZEB2 was associated with an epilepsy drug therapy.

Conclusion: Our findings suggest that *ZEB2* may be involved in the therapy of epilepsy, and rs10496964 regulates *ZEB2* expression in human brain tissue.

Keywords

ZEB2, epilepsy, therapy, biomarker, rs10496964, protein–protein interaction, bioinformatic analysis, expression quantitative trait loci, drug target, histone deacetylase

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Introduction

The epilepsies are a group of brain disorders that affect up to 4% of all people at some time in their lives.[1] The current treatments for epilepsy are largely unsatisfactory,[2] mainly as a result of the unclear pathogenesis of these disorders. Clinical genetic data about the common epilepsies indicate complex inheritance, and genetic approaches are thus likely to be important for understanding at least some mechanisms of epilepsy pathogenesis. In addition, some evidence suggests a role for gene polymorphisms in epilepsy,[3–5] but their contributions remain controversial, mainly because of relatively small sample sizes and a lack of functional validation of these polymorphisms.

A genome-wide association study has revealed a significant association between epilepsy and the rs10496964 polymorphism.[6] This polymorphism is located in an intergenic region that is nearest to the *ZEB2* gene, which encodes the zinc finger E-box binding homeobox 2 (ZEB2) protein. Interestingly, polymorphisms in noncoding regions may confer disease risk by regulating the expression of a target gene.[7] However, the relationship between rs10496964 and *ZEB2* expression has not yet been evaluated. A previous study explored the relationship between *ZEB2* and epilepsy, but did not find a role for *ZEB2* in epilepsy treatment, and did not find that the rs13020210 polymorphism regulates the expression of *ZEB2*.[8] Mutations in *ZEB2* are associated with Hirschsprung disease/Mowat–Wilson syndrome.[9,10] Epilepsy is one of the main features of Mowat–Wilson syndrome in most patients,[11] suggesting common pathogenic mechanisms between the two conditions. However, although genetic analyses have provided important information about the pathogenesis of epilepsy, such data remain difficult to explain.

To further evaluate the role of *ZEB2* in epilepsy treatment and explore the association between rs10496964 and *ZEB2* expression, we conducted expression quantitative trait loci (eQTL) analysis, pathway and process enrichment analysis, protein–protein interaction (PPI) analysis, and PhosphoSitePlus® analysis.

Materials and methods

Ethics and consent

This study consisted of a bioinformatic analysis that did not involve humans or animals. Therefore, local ethics committee approval and informed consent were not required.

eQTL analysis

Previous studies have indicated that most disease-associated polymorphisms confer disease risk by acting as eQTL to regulate gene expression.[12–15] Considering that rs10496964 is an intergenic variant, we performed an eQTL analysis to evaluate the association between this polymorphism and *ZEB2* expression in human brain tissue because epilepsy is a chronic brain disease. The association between the rs10496964 genotype and *ZEB2* expression was assessed using a linear regression analysis under an additive model in the brain tissue. We selected the eQTL dataset

resource from the Brain eQTL Almanac, which consists of 10 datasets of tissue from 10 brain regions, from 134 individuals.[16] This resource contains datasets from tissue from the following brain regions: frontal cortex, temporal cortex, occipital cortex, putamen, substantia nigra, medulla, hippocampus, thalamus, cerebellum, and white matter.

Pathway and process enrichment analysis

Gene enrichment analysis is an effective tool to apply to the analysis and interpretation of biological data. We used this tool to discover the shared functions or properties of the biological items represented within lists of genes. This method can provide important biological insights and reveal participation in the same biological activities or pathways associated with a disease. We carried out pathway and process enrichment analysis using the Metascape database to investigate the genes that are co-expressed with *ZEB2*.[17] Metascape is a tool designed to provide comprehensive gene list annotations. It is an analytical resource, with the integration of a large number of current biological databases, and is a robust analytical pipeline.

PPI analysis

Information from PPI network analysis is beneficial for understanding disease associations in detail. To evaluate the role of *ZEB2* in drug treatments, we curated the targets of approved drugs for epilepsy using two databases: DrugBank 5.0[18] and the Therapeutic Target Database 2020.[19] We also investigated the interactions between ZEB2 and the proteins encoded by likely epilepsy-related genes. The PPIs were evaluated using the STRING database (https://string-db.org/cgi/input.pl), which presents known and predicted PPI.[20] We then used Cytoscape software to construct PPI networks.[21]

PhosphoSitePlus® analysis

Protein modifications and their regulation are associated with protein function. Proteins are the most common biological molecules, and perform a vast array of biological functions within living organisms. By controlling the modifications of protein surfaces, these biological molecules can be re-engineered to provide the desired functions of biomolecule detection, assay, tracking, or targeting. We conducted a PhosphoSitePlus® analysis of ZEB2 to further investigate its potential in epilepsy therapy.

Results

eQTL analysis

The rs10496964 T allele was associated with *ZEB2* expression in tissue from both the temporal cortex and the putamen ($P = 0.0093$ and 0.027, respectively) (Figure 1).

Pathway and process enrichment analysis

We identified 625 genes that are co-expressed with *ZEB2* by scanning the COEXPEDIA database.[22] The sum of their edges' log-likelihood scores was greater than one point. Next, we performed pathway and process enrichment analysis of these co-expressed genes and *ZEB2* using the Metascape database, to identify the possible biological pathways of *ZEB2* in epilepsy. This analysis revealed that a large number of the biological pathways were associated with infection and inflammation (Figure 2).

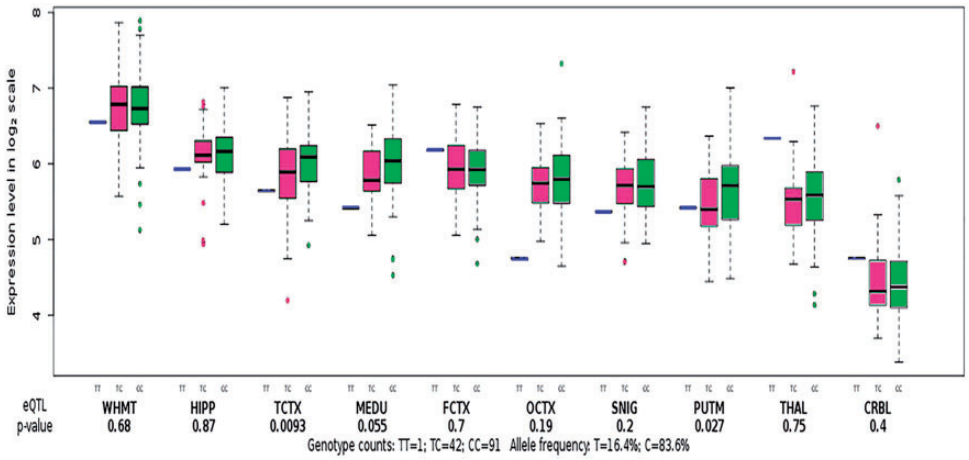

Figure 1. Rs10496964 is an expression quantitative trait locus (eQTL) that affects *ZEB2* expression in human brain tissue. Association between the rs10496964 genotype and *ZEB2* expression using linear regression analysis under an additive model in each of the 10 human brain tissue regions. Data were retrieved from the Brain eQTL Almanac database.

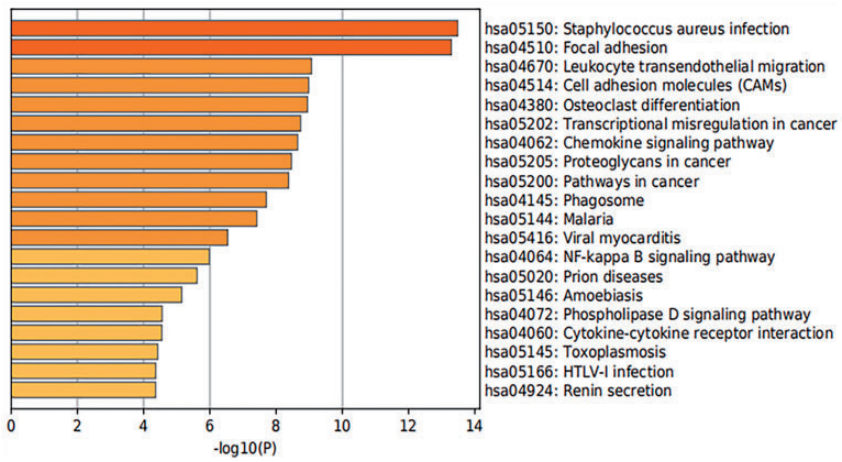

Figure 2. Pathway and process enrichment analysis. Bar graph of the enriched terms across input gene lists, colored by *P*-values. *P*-values were calculated based on the accumulative hypergeometric distribution.

PPI analysis

To evaluate the role of *ZEB2* in drug repositioning, we obtained 115 genes that are targeted by epilepsy drugs from two drug target databases (DrugBank 5.0 and the Therapeutic Target Database 2020). The results of PPI analysis demonstrated that ZEB2 interacts with epilepsy drug targets (Figure 3a). Information about these genes is shown in Table 1. We also identified 84 genes that are considered to be epilepsy-related genes, 73 genes associated with both brain development malformations and epilepsy, and 536 genes associated with both physical or other systemic

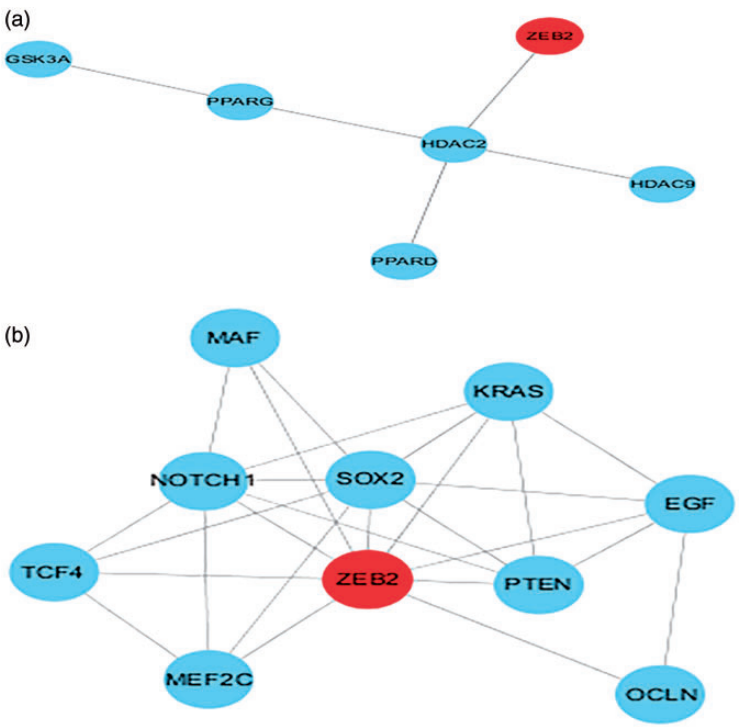

Figure 3. Protein–protein interaction (PPI) networks. (a) PPI networks of the proteins encoded by *ZEB2* and the genes targeted by approved epilepsy drugs. The red node represents *ZEB2* and the blue nodes indicate genes targeted by approved epilepsy drugs. (b) PPI networks of the proteins encoded by *ZEB2* and likely epilepsy genes. The red node represents *ZEB2* and the blue nodes indicate likely epilepsy genes.

Table 1. Information about the genes encoding antiepileptic drug targets.

Gene	Genomic location	Encoded protein	Post-translational modifications	Epilepsy drug
GSK3A	Chr19	Glycogen synthase kinase-3 alpha	Phosphorylation, acetylation, ubiquitylation, and other	Valproate
PPARG	Chr3	Peroxisome proliferator-activated receptor gamma	Phosphorylation, acetylation, ubiquitylation, and other	Valproate
HDAC2	Chr6	Histone deacetylase 2	Phosphorylation, acetylation, ubiquitylation, and other	Valproate
PPARD	Chr6	Peroxisome proliferator-activated receptor delta	Phosphorylation, ubiquitylation, and other	Valproate
HDAC9	Chr7	Histone deacetylase 9	Phosphorylation and ubiquitylation	Valproate

Table 2. Information about epilepsy genes whose encoded proteins interact with the ZEB2 protein.

Gene	Genomic location	Encoded protein	Post-translational modifications	Related diseases
EGF	Chr4	Pro-epidermal growth factor	Phosphorylation, ubiquitylation, and other	Epilepsy or seizures
KRAS	Chr12	GTPase KRas	Phosphorylation, acetylation, ubiquitylation, and other	Epilepsy or seizures
MAF	Chr16	Transcription factor Maf	Phosphorylation, ubiquitylation, and other	Epilepsy or seizures
MEF2C	Chr5	Myocyte-specific enhancer factor 2C	Phosphorylation, acetylation, and other	Epilepsy or seizures
NOTCH1	Chr9	Neurogenic locus notch homolog protein 1	Phosphorylation, acetylation, ubiquitylation, and other	Epilepsy or seizures
PTEN	Chr10	Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN	Phosphorylation, acetylation, ubiquitylation, and other	Epilepsy or seizures
SOX2	Chr3	Transcription factor SOX-2	Phosphorylation, acetylation, ubiquitylation, and other	Epilepsy or seizures
TCF4	Chr18	Transcription factor 4	Phosphorylation, acetylation, ubiquitylation, and other	Epilepsy or seizures
OCLN	Chr5	Occludin	Phosphorylation and ubiquitylation	Brain development malformations and epilepsy

abnormalities and epilepsy or seizures.[23] The results of PPI analysis showed that ZEB2 interacts with the proteins encoded by nine epilepsy genes (Figure 3b). Information about these genes is shown in Table 2.

PhosphoSitePlus® analysis

PhosphoSitePlus® analysis of ZEB2 revealed four types of modifications: phosphorylation, acetylation, ubiquitylation, and other. Phosphorylation, ubiquitylation, and other were present, but were not associated with any specific condition. For the K1150 acetylation site modification, the condition was also unclear. However, our analysis revealed that histone deacetylase (HDAC) is linked to the K377 acetylation site (Figure 4).

Discussion

Although drug treatment has evolved rapidly in recent years, approximately 30% of patients still suffer from recurrent seizures, resulting in a medically severe and socially disabling condition.[24,25] However, the personalization of treatments targeted toward the precise molecular pathogenesis of an illness[26,27] may be able to avoid such conditions in the future. A previous study did not find *ZEB2* to be a potential target for epilepsy treatment, and did not identify any variants regulating *ZEB2* expression.

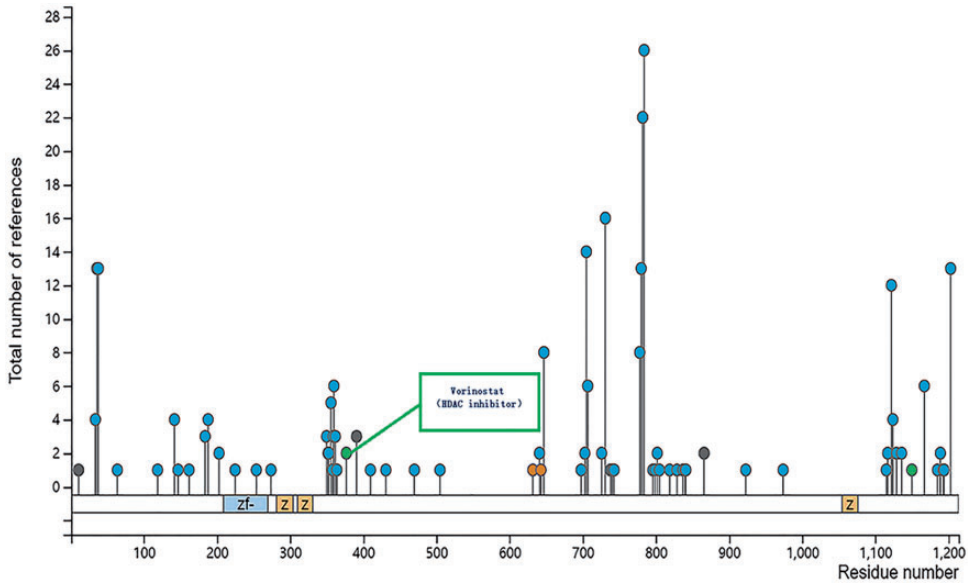

Figure 4. Phosphoproteomic bioinformatic analysis of ZEB2 protein. PhosphoSitePlus® analysis revealed that histone deacetylase (HDAC) is linked to the K377 acetylation site.

This may be because the authors of this previous study did not fully integrate the data from a large number of databases.[8] In the present study, we integrated data from a brain tissue eQTL analysis, pathway and process enrichment analysis, PPI analysis, and PhosphoSitePlus® analysis, and first identified that *ZEB2* may be involved in epilepsy drug therapy. We also revealed that the rs10496964 polymorphism regulates the expression of *ZEB2* in human brain tissue. To the best of our knowledge, this is the most comprehensive study to have explored the role of *ZEB2* in epilepsy.

The rs10496964 polymorphism is located in an intergenic region, and is nearest to *ZEB2*. Currently, the underlying association between rs10496964 and *ZEB2* is unknown. Although regulatory elements are present in the intergenic regions of genes, they can also regulate gene expression at a great distance from the target gene.[28] To investigate the functional link between *ZEB2* and rs10496964 at a molecular level, we performed eQTL analysis to assess whether the rs10496964 genotype was significantly associated with *ZEB2* transcript expression in human brain tissue. Using an eQTL dataset in human brain tissue, the rs10496964 T allele was associated with lower *ZEB2* expression in both the temporal cortex and putamen. However, we did not find any evidence that rs10496964 modulated *ZEB2* expression in the frontal cortex, occipital cortex, substantia nigra, medulla, hippocampus, thalamus, cerebellum, or white matter. The main reason for this finding may be that rs10496964 regulates *ZEB2* expression in a region-specific manner in the human brain. Notably, changes in the temporal cortex and putamen have been reported to be often associated with seizures.[29,30]

Pathway and process enrichment analysis revealed that the significantly enriched pathways can be mainly divided into three classes: pathways associated with cancer (transcriptional misregulation in cancer,

proteoglycans in cancer, and pathways in cancer), pathways associated with fundamental cellular processes (osteoclast differentiation, focal adhesion, cell adhesion molecules, and phospholipase D signaling pathway), and pathways associated with infection and inflammation (*Staphylococcus aureus* infection, leukocyte transendothelial migration, chemokine signaling pathway, phagosome, malaria, viral myocarditis, nuclear factor kappa-light-chain-enhancer of activated B cells [NF-κB] signaling pathway, prion diseases, amoebiasis, toxoplasmosis, cytokine–cytokine receptor interaction, and human T-cell leukemia virus type 1 [HTLV-1] infection). Among these enrichment pathways, the largest number of pathways were associated with infection and inflammation. The inflammatory pathway is thought to play a vital role in the development of epilepsy.[31] Furthermore, increasing evidence suggests that inflammatory pathways might be related to several other neuropsychiatric comorbidities, including cognitive dysfunction,[32,33] depression,[34,35] autism spectrum disease,[36] anxiety,[37] and schizophrenia.[38]

The investigation of protein–protein networks can be used for drug target discovery, drug discovery, and drug design. This method is currently very important because it helps to elucidate the route that transforms a biological network into an illness pathway. This new method is therefore likely to be very effective for dealing with complex diseases.[39–41] To obtain a basic understanding of an illness, PPI networks show the associations between nodes from a global viewpoint. PPI networks are also beneficial for understanding disease progression.[42,43] In the current study, PPI network analysis revealed that ZEB2 interacts with a number of targets of epilepsy drugs. We also found that ZEB2 interacts with the proteins encoded by nine epilepsy-related genes. A PPI network indicates an association between proteins in a biological pathway.[44] Therefore, we can reasonably speculate that *ZEB2* may be involved in epilepsy, and might thus be a potential therapeutic target for this disorder.

Our PhosphoSitePlus® analysis revealed that HDAC is linked to ZEB2 acetylation. HDAC is a family of enzymes that are associated with the epigenetic modulation of genomic activity.[45,46] Dysregulation of their activity can result in many neurological diseases.[47,48] HDAC inhibitors may be an effective treatment for brain disorders, including epilepsy, because they can increase the acetylation of histones, maintain the balance of histone acetylation, and correct transcriptional dysfunction.[49–52] Valproic acid is a broad anti-seizure drug that is a first-line treatment for epilepsy. However, valproic acid has also been reported to inhibit HDACs.[53] Recently, a phenotypic screening platform found that HDAC inhibition is likely an effective treatment for epilepsy.[54] Early treatment with HDAC inhibitors might thus be an effective strategy for preventing epileptogenesis, as well as for reducing behavioral comorbidities.[55] Given that HDAC plays a key role in epilepsy treatment, *ZEB2* may be a potential therapeutic target for treating epilepsy.

In conclusion, we identified that *ZEB2* may be involved in the treatment of epilepsy and that rs10496964 regulates the expression of *ZEB2* in human tissue, via an integrative analysis involving eQTL analysis, pathway and process enrichment analysis, PPI analysis, and PhosphoSitePlus® analysis.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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