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

Shitao Wang¹, Dan Wang¹, Xuemei Cai², Qian Wu¹ and Yanbing Han¹

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

¹Department of Neurology, First Affiliated Hospital of Kunming Medical University, Kunming, China
²Department of Clinical Laboratory, First Affiliated Hospital of Kunming Medical University, Kunming, China

Corresponding author:
Yanbing Han, Department of Neurology, The First Affiliated Hospital of Kunming Medical University, 295 Xi Chang Road, Kunming, Yunnan 650032, China.
Email: ynhyb@163.com
Introduction

The epilepsies are a group of brain disorders that affect up to 4% of all people at some time in their lives. The current treatments for epilepsy are largely unsatisfactory, 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, 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. 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. 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. Mutations in ZEB2 are associated with Hirschsprung disease/Mowat–Wilson syndrome. Epilepsy is one of the main features of Mowat–Wilson syndrome in most patients, 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. 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 5.018 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).
To evaluate the role of ZEB2 in drug reposi-
tioning, we obtained 115 genes that are tar-
geted 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 1. Rs10496964 is an expression quantiative 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 reposi-
tioning, we obtained 115 genes that are tar-
geted 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     |
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. However, the personalization of treatments targeted toward the precise molecular pathogenesis of an illness 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.

| 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 |
This may be because the authors of this previous study did not fully integrate the data from a large number of databases. 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. 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.

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.

Acknowledgements
We thank Sunbing Du and Wenqiu Yang for software assistance.

Declaration of conflicting interest
The authors declare that there is no conflict of interest.
Funding
The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was funded by the National Natural Science Foundation of China (grant no. 81260199, 81660228, and 81601134), the Yunnan Province Talent Training Program (grant no. 2017HB048 and H-2018056), and the Yunnan Science and Research Funding Program (grant no. 2017FA041, 2018HC008, 2016NS029, and 2017FE468(-144)).

ORCID iD
Yanbing Han https://orcid.org/0000-0002-6731-1335

References
1. Hesdorffer DC, Logroscino G, Benn EK, et al. Estimating risk for developing epilepsy: a population-based study in Rochester, Minnesota. *Neurology* 2011; 76: 23–27.
2. Simonato M, Brooks-Kayal AR, Engel J, et al. The challenge and promise of antiepileptic therapy development in animal models. *Lancet Neurol* 2014; 13: 949–960.
3. Abou EI Ella SS, Tawfik MA, Abo El Fotoh WMM, et al. The genetic variant “C588T” of GABARG2 is linked to childhood idiopathic generalized epilepsy and resistance to antiepileptic drugs. *Seizure* 2018; 60: 39–43.
4. Butil/A T, Zazgyva A, Sin AI, et al. GABRG2 C588T gene polymorphisms might be a predictive genetic marker of febrile seizures and generalized recurrent seizures: a case-control study in a Romanian pediatric population. *Arch Med Sci* 2018; 14: 157–166.
5. Bhat MA, Guru SA, Mir R, et al. Association of GABAA receptor gene with epilepsy syndromes. *J Mol Neurosci* 2018; 65: 141–153.
6. Chou IC, Lee CC, Tsai CH, et al. Genome-wide association analysis of genetic generalized epilepsies implicates susceptibility loci at 1q43, 2p16.1, 2q22.3 and 17q21.32. *Hum Mol Genet* 2012; 21: 5359–5372.
7. Li X, Luo Z, Gu C, et al. Common variants on 6q16.2, 12q24.31 and 16p13.3 are associated with major depressive disorder. *Neuropsychopharmacology* 2018; 43: 2146–2153.
8. International League Against Epilepsy Consortium on Complex Epilepsies. Genome-wide mega-analysis identifies 16 loci and highlights diverse biological mechanisms in the common epilepsies. *Nat Commun* 2018; 9: 5269.
9. Mowat DR, Wilson MJ and Goossens M. Mowat-Wilson syndrome. *J Med Genet* 2003; 40: 305–310.
10. Paz JA, Kim CA, Goossens M, et al. Mowat-Wilson syndrome: neurological and molecular study in seven patients. *Arq Neuropsiquiatr* 2015; 73: 12–17.
11. Garavelli L, Zollino M, Mainardi PC, et al. Mowat–Wilson syndrome: facial phenotype changing with age: study of 19 Italian patients and review of the literature. *Am J Med Genet A* 2009; 149A: 417–426.
12. Nicolae DL, Gamazon E, Zhang W, et al. Trait-associated SNPs are more likely to be eQTLs: annotation to enhance discovery from GWAS. *PLoS Genet* 2010; 6: e1000888.
13. Guo X, Lin W, Bao J, et al. A comprehensive cis-eQTL analysis revealed target genes in breast cancer susceptibility loci identified in genome-wide association studies. *Am J Hum Genet* 2018; 102: 890–903.
14. Ward LD and Kellis M. Interpreting non-coding genetic variation in complex traits and human disease. *Nat Biotechnol* 2012; 30: 1095–1106.
15. Maurano MT, Humbert R, Rynes E, et al. Systematic localization of common disease-associated variation in regulatory DNA. *Science* 2012; 337: 1190–1195.
16. Ramasamy A, Trabzuni D, Guelfi S, et al. Genetic variability in the regulation of gene expression in ten regions of the human brain. *Nat Neurosci* 2014; 17: 1418–1428.
17. Zhou Y, Zhou B, Pache L, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun* 2019; 10: 1523.
18. Wishart DS, Feunang YD, Guo AC, et al. DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res* 2018; 46: D1074–D1082.
19. Wang Y, Zhang S, Li F, et al. Therapeutic target database 2020: enriched resource for facilitating research and early development of targeted therapeutics. *Nucleic Acids Res* 2020; 48: D1031–D1041.

20. Von Mering C, Huynen M, Jaeggi D, et al. STRING: a database of predicted functional associations between proteins. *Nucleic Acids Res* 2003; 31: 258–261.

21. Su G, Morris JH, Demchak B, et al. Biological network exploration with Cytoscape 3. *Curr Protoc Bioinformatics* 2014; 47: 1–24.

22. Yang S, Kim CY, Hwang S, et al. COEXPEDIA: exploring biomedical hypotheses via co-expressions associated with medical subject headings (MeSH). *Nucleic Acids Res* 2017; 45: D389–D396.

23. Wang J, Lin ZJ, Liu, L, et al. Epilepsy-associated genes. *Seizure* 2017; 44: 11–20.

24. Symonds JD, Zuberi SM and Johnson MR. Advances in epilepsy gene discovery and implications for epilepsy diagnosis and treatment. *Curr Opin Neurol* 2017; 30: 193–199.

25. Kearney H, Byrne S, Cavalleri GL, et al. Tackling epilepsy with high-definition precision medicine: a review. *JAMA Neurol* 2019.

26. Orsini A, Esposito M, Perna D, et al. Personalized medicine in epilepsy patients. *J Transl Genet Genom* 2018; 2: 16.

27. Striano P, Vari MS, Mazzocchetti C, et al. Management of genetic epilepsies: from empirical treatment to precision medicine. *Pharmacol Res* 2016; 107: 426–429.

28. Ometto L, Stephan W and De Lorenzo D. Insertion/deletion and nucleotide polymorphism data reveal constraints in Drosophila melanogaster introns and intergenic regions. *Genetics* 2005; 169: 1521–1527.

29. MacEachern SJ, Santoro JD, Hahn KJ, et al. Children with epilepsy demonstrate macro- and microstructural changes in the thalamus, putamen, and amygdala. *Neuroradiology* 2020; 62: 389–397.

30. Stefanits H, Milenkovic I, Mahr N, et al. Alterations in GABAA receptor subunit expression in the amygdala and entorhinal cortex in human temporal lobe epilepsy. *J Neuropathol Exp Neurol* 2019; 78: 1022–1048.

31. Vezzani A, Lang B and Aronica E. Immunity and inflammation in epilepsy. *Cold Spring Harb Perspect Med* 2015; 6: a022699.

32. Marsland AL, Gianaros PJ, Kuan DC, et al. Brain morphology links systemic inflammation to cognitive function in midlife adults. *Brain Behav Immun* 2015; 48: 195–204.

33. McAfoose J and Baune B. Evidence for a cytokine model of cognitive function. *Neurosci Biobehav Rev* 2009; 33: 355–366.

34. Krishnadas R and Cavanagh J. Depression: an inflammatory illness? *J Neurol Neurosurg Psychiatry* 2012; 83: 495–502.

35. Slavich GM and Irwin MR. From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. *Psychol Bull* 2014; 140: 774.

36. Mazarati AM, Lewis ML and Pittman QJ. Neurobehavioral comorbidities of epilepsy: role of inflammation. *Epilepsia* 2017; 58: 48–56.

37. Vogelzangs N, Beekman A, De Jonge P, et al. Anxiety disorders and inflammation in a large adult cohort. *Transl Psychiatry* 2013; 3: e249.

38. Muller N, Weidinger E, Leitner B, et al. The role of inflammation in schizophrenia. *Front Neurosci* 2015; 9: 372.

39. Csermely P, Agoston V and Pongor S. The efficiency of multitarget drugs: the network approach might help drug design. *Trends Pharmacol Sci* 2005; 26: 178–182.

40. Dancey JE and Chen HX. Strategies for optimizing combinations of molecularly targeted anticancer agents. *Nat Rev Drug Discov* 2006; 5: 649–659.

41. Zimmermann GR, Lehár J and Keith CT. Multi-target therapeutics: when the whole is greater than the sum of the parts. *Drug Discov Today* 2007; 12: 34–42.

42. Ideker T, Ozyer O, Schwikowski B, et al. Discovering regulatory and signalling circuits in molecular interaction networks. *Bioinformatics* 2002; 18: S233–S240.

43. Hallock P and Thomas MA. Integrating the Alzheimer’s disease proteome and transcriptome: a comprehensive network model of a complex disease. *OMICS* 2012; 16: 37–49.

44. Chakraborty C, Doss C GP, Chen L, et al. Evaluating protein-protein interaction (PPI)
networks for diseases pathway, target discovery, and drug-design using ‘in silico pharmacology’. *Curr Protein Pept Sci* 2014; 15: 561–571.

45. De Ruijter AJM, Van Gennip AH, Caron HN, et al. Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem J* 2003; 370: 737–749.

46. Qiu X, Xiao X, Li N, et al. Histone deacetylases inhibitors (HDACis) as novel therapeutic application in various clinical diseases. *Prog Neuro-Psychopharmacol Biol Psychiatry* 2017; 72: 60–72.

47. Penney J and Tsai LH. Histone deacetylases in memory and cognition. *Sci Signal* 2014; 7: re12.

48. Landgrave-Gómez J, Mercado-Gómez O and Guevara-Guzmán R. Epigenetic mechanisms in neurological and neurodegenerative diseases. *Front Cell Neurosci* 2015; 9: 58.

49. Chuang DM, Leng Y, Marinova Z, et al. Multiple roles of HDAC inhibition in neurodegenerative conditions. *Trends Neurosci* 2009; 32: 591–601.

50. Abel T and Zukin RS. Epigenetic targets of HDAC inhibition in neurodegenerative and psychiatric disorders. *Curr Opin Pharmacol* 2008; 8: 57–64.

51. Kazantsev AG and Thompson LM. Therapeutic application of histone deacetylase inhibitors for central nervous system disorders. *Nat Rev Drug Discov* 2008; 7: 854–868.

52. O’Connor SS, Jobes DA, Lineberry TW, et al. An investigation of emotional upset in suicide ideation. *Arch Suicide Res* 2010; 14: 35–43.

53. Gottlicher M, Minucci S, Zhu P, et al. Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. *EMBO J* 2001; 20: 6969–6978.

54. Ibhazehiebo K, Gavrilovici C, De La Hoz CL, et al. A novel metabolism-based phenotypic drug discovery platform in zebrafish uncovers HDACs 1 and 3 as a potential combined anti-seizure drug target. *Brain* 2018; 141: 744–761.

55. Citraro R, Leo A, De Caro C, et al. Effects of histone deacetylase inhibitors on the development of epilepsy and psychiatric comorbidity in WAG/Rij rats. *Mol Neurobiol* 2020; 57: 408–421.