ME2 association analysis in adolescent onset genetic generalized epilepsies

To the Editors:
I have read with interest the article “Replication, reanalysis, and gene expression: ME2 and genetic generalized epilepsy” by Wang et al. The authors present a follow-up association and expression analysis of their initial report suggesting that genetic variation of the gene encoding the malic enzyme 2 (ME2) may underlie susceptibility to adolescent onset genetic generalized epilepsies (GGEado). Our previous replication analysis failed to support the initial association claim. In their follow-up study, Wang et al carried out a replication association analysis of 11 ME2 single nucleotide polymorphisms (SNPs) in newly recruited GGEado cohorts of Caucasian ancestry. They found a strong association of SNP rs608781 with GGEado (POPFAM+ P = .0006). Remarkably, this association was not supported by three highly correlated ME2 SNPs (rs625566, P = .135; rs605902, P = .185; rs649224, P = .051). Due to their complete linkage disequilibrium (LD; R² = 1.00; Table S2), these four LD-proxy SNPs virtually represent genotyping replicates for which the association statistics should generate essentially the same P values. Accordingly, the strongly deviating P values of these LD-proxy SNPs may indicate a spurious association of ME2 SNP rs608781 with GGEado.

Wang et al also presented an association analysis of the ME2 three-SNP (rs2850545-rs645088-rs649224) C-C-C risk haplotype by reanalyzing the previously collected Caucasian GGEado cohorts in comparison with 503 European reference samples from the 1000 Genomes Project to control for confounding by population structure. Their refined reanalysis confirmed a significant association of the ME2 three-SNP risk haplotype with GGEado (POPFAM+ P = .002). Taking into account the substantial overlap of the GGEado cohorts and the high correlation between the ME2 three-SNP risk haplotype and the initial nine-SNP risk haplotype, the reported three-SNP haplotype association largely reflects the previous association claim and adds little replication evidence. This brings up the question of why haplotype association analysis was not performed in the new replication cohorts, given that the available 11 ME2 SNPs are suitable for tagging the common ME2 three-SNP risk haplotype.

To test the validity of the reported ME2 associations, we have carried out replication analyses in 874 unrelated European subjects with GGEado and 3893 unrelated European ancestry-matched control subjects (EPICURE GGE genome-wide association study [GWAS] case-control cohorts; see Appendix S1) using the same ascertainment scheme reported by Greenberg et al. We failed to replicate the association of GGEado with ME2 SNP rs608781 (logistic regression, P = .172; OR(T) = 0.869, 95% confidence interval = 0.71-1.063) and the ME2 C-C-C risk haplotype (P = .478; Table S1). Likewise, the genotype frequencies for SNP rs608781 found in the EPICURE GGEado subjects were similar to those observed in the 503 European controls used by Wang et al (χ² = 0.39, 2 df, P = .824). Overall, none of the 11 ME2 SNPs investigated by Wang et al showed evidence for an association with GGEado (logistic regression, P > .17; Table S1). Taken together, the inconsistent replication results presented by Wang et al and our replication failure do not support evidence that genetic variation of ME2 increases risk of GGEado.

ACKNOWLEDGMENTS
The author thanks all clinical partners of the EPICURE Consortium contributing to the collection of the EPICURE GGE GWAS cohort (see Appendix S1). This work was supported by the German Research Foundation Research Unit FOR 2715 (grant SA434/6-1) and the European Union FP6 Integrated Project EPICURE (grant LSHM-CT-2006-037315).

CONFLICT OF INTEREST
The author has no conflict of interest to disclose. I confirm that I have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.
REFERENCES


SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.
In Response: ME2 association analysis in adolescent-onset genetic generalized epilepsy

To the Editors:
Dr. Sander asserts that our replication results are inconsistent, despite our reporting three separate lines of evidence: ME2 gene expression, association, and replication; all supporting our finding that ME2 (malic enzyme 2) influences genetic generalized epilepsy (GGE).

In 1000 Genomes, the correlation between selected ME2 single nucleotide polymorphisms (SNPs) is high (Figure 1A). Dr. Sander assumes that the correlation should be high in our GGE cases too. On the basis of this assumption, he concludes that the evidence for association (ie, P-values) at these selected ME2 SNPs should be the same. However, these SNPs are not all highly correlated in our GGE cases (Figure 1B). Specifically, the SNP with the smallest P-value, rs608781, is not highly correlated ($r^2 = 0.48$) with any of the other ME2 SNPs. Moreover, cases and controls may well have different patterns of correlation at associated SNPs. Therefore, our replication results are not inconsistent as Sander claims.

FIGURE 1 A, High correlation between selected ME2 single nucleotide polymorphisms (SNPs; including rs608781) for 1000 Genomes reference samples of European ancestry (EUR). B, Relatively low correlation between rs608781 and the remaining ME2 SNPs in our genetic generalized epilepsy (GGE) cases.

Given that our replication results are consistent and supported by other evidence, the more relevant question is: How do our cases differ from Sander’s cases? It is likely that our GGE cases are more homogeneous than Sander’s cases because our cases were rigorously phenotyped, and many had a positive family history of disease. This means that our cases are more likely to have the same basis for GGE, and if that basis is related to genetic variation at ME2, then we should (in principle) see a difference between cases and controls. By contrast, Sander’s sample of GGE cases ($n = 3893$) likely contains increased heterogeneity due to diagnostic variation across centers and genetic differences in population substructure. This means his cases likely have GGE for different genetic reasons (ie, heterogeneity is present). As we have written elsewhere, heterogeneity is the most likely reason that a genetic study misses a true signal. For example, despite the high heritability of GGE (65%-80%), the reason that GGE genes have not been convincingly found by large-scale
genetic studies is likely heterogeneity. Worse yet, given the aforementioned misuse of \( P \)-values, any data set containing such heterogeneity could be used to “refute” any published findings done elsewhere.

**CONFLICT OF INTEREST**

The authors declare no conflict of interests. The authors confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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**REFERENCES**

3. Greenberg DA, Stewart WL. Remind me again what disease we are studying? A population genetics, genetic analysis, and real data perspective on why progress on identifying genetic influences on common epilepsies has been so slow. Prog Brain Res. 2014;213:199–221.
To the Editors:

Common symptoms of focal seizures include hot or cold skin sensations or a “rising sense” through the body. Both of these are sometimes, but not always, mediated by the autonomic nervous system. The 2017 International League Against Epilepsy (ILAE) classification of seizures does not clarify whether these sensations indicate a focal autonomic or focal sensory seizure. Therefore, the ILAE task force on classification of seizures has issued the following clarification.

Some sensations, particularly a rising sensation or diffuse hot-cold sensations, may be classified either as autonomic or as sensory. These symptoms often are associated with other autonomic symptoms or signs, such as nausea, vomiting, flushing, piloerection, or palpitations, marking them clearly as autonomic. In the absence of accompanying autonomic symptoms, it is acceptable to classify the seizure as either focal autonomic or focal sensory, depending upon the clinical context.

CONFLICT OF INTEREST

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Robert S. Fisher1 12
Helen Cross2
Carol D’Souza3
Jacqueline A. French4 40
Sheryl Haut5
Norimichi Higurashi6
Edouard Hirsch7
Floor E. Jansen8
Jukka Peltola9,10
Solomon L. Moshe11
Emilio Perucca12 13
Lieven Lagae13
Eliane Roulet-Perez14
Andreas Schulze-Bonhage15
Ingrid E. Scheffer16
Ernest Somerville17 18
Michael R. Sperling18
Samuel Wiebe19 20
Elza Marcia Yacubian20
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REFERENCES


Announcements

2nd International Congress on Mobile Devices and Seizure Detection in Epilepsy
6–7 September 2019
Lausanne, Switzerland
http://www.mhsdepilepsy2019.com/

4th International Epilepsy Symposium: Epilepsy and Psychology
Seizures, Cognition, and Behavior
6–7 September 2019
Bielefeld, Germany
Information: https://www.ilae.org/congresses/4th-international-epilepsy-symposium-epilepsy-and-psychology

4th International Symposium on Hypothalamic Hamartomas
12–14 September 2019
Washington, D.C., USA
Symposium website: http://www.hopeforrh.org/4th-international-symposium-on-hypothalamic-hamartomas/

Cleveland Clinic Neurological Institute Summit 2019: Epilepsy - Focal Cortical Displasia
12–15 September 2019
Cleveland, OH, US
Website: http://www.clevelandclinicmeded.com/live/courses/ni-summit-epilepsy/default.asp

ILAE British Branch 17th SpR Epilepsy Teaching Weekend
14–15 September 2019
The Mathematics Institute in Oxford, UK.
http://www.epilepsyteachingweekend.com/

Introduction to Neuropsychological Methods in the Diagnosis and Treatment of People with Epilepsy
18–22 September 2019

Hanoï, Vietnam

Congreso LACE
19–20 September 2019
Buenos Aires, Argentina
http://www.lace.org.ar/constructor.php?categoria=1

9th Migrating Course on Epilepsy
19–22 September 2019
Vrdnik, Serbia
Information: https://www.ilae.org/congresses/9th-migrating-course-on-epilepsy

Canadian League Against Epilepsy 2019 Annual Scientific Meeting
20–22 September 2019
Winnipeg, Manitoba
https://claegroup.org/2019-meeting

36ta Conferencia Epilepsia del Caribe
21 September 2019
San Juan, Puerto Rico, USA
Information: https://www.ilae.org/congresses/36ta-conferencia-epilepsia-del-caribe

Philippine League Against Epilepsy 10th Biennial Epilepsy Congress: Epilepsy Across the Ages: Advancing the Science, Improving the Care
26–28 September 2019
Manilla, Philippines
Congress Programme

Masterclass on Resistant Epilepsy – Part 2
2 October 2019
Bucharest, Romania
Information: https://www.ilae.org/congresses/masterclass-on-resistant-epilepsies-m2

**2019 ILAE British Branch Annual Scientific Meeting**

2–4 October 2019
Birmingham, UK
http://www.ilaebritishconference.org.uk/

**Park City Epilepsy Meeting: Cutting Edge Approaches to Transform Epilepsy Therapy**

6–8 October 2019
Utah, USA
Website: http://www.parkcityepilepsymeeting.com/

**European Congress of NeuroRehabilitation 2019 (ECNR)**

9–12 October 2019
Budapest, Hungary
https://www.ecnr-congress.org/

**Jahrestagung des deutsch-österreichisch-schweizer Arbeitskreises Epilepsie**

**German-Austrian-Swiss Workshop on Epileptology (DACH)**

10–12 October 2019
Friedrichshafen, Germany
https://www.uniklinik-freiburg.de/jahrestagung-dach-ag-epilepsie.html

**9th Caucasian Summer School on Clinical Epileptology**

11–13 October 2019
Tbilisi, Georgia
Information: https://www.ilae.org/congresses/9th-caucasian-summer-school-on-clinical-epileptology-cssce-ix

**EAN Autumn School 2019**

17–20 October 2019
Loutraki, Greece
https://www.ean.org/Autumn-School.3752.0.html

**ISPN 2019: 47th Annual Meeting of the International Society for Pediatric Neurology**

20–24 October 2019
ICC Birmingham, Birmingham, UK
https://www.ispnmeeting.org/2019/

**Epilepsy and Psychiatric Disorders throughout Life**

**Educational Symposium of the Psychiatry Commission**

25–26 October 2019
São Paulo, Brazil
Information: https://www.ilae.org/congresses/epilepsy-and-psychiatric-disorders-throughout-life

**WCN 2019: XXIV World Congress of Neurology**

27–31 October 2019
Dubai, United Arab Emirates
https://2019.wcn-neurology.com/

**Epilepsy Society of Australia 33rd Annual Scientific Meeting**

6–8 November 2019
Sydney, Australia

**Congreso Argentino de Neurología**

19–22 November 2019
Mar del Plata, Argentina

**2nd MAGNIMS-ESNR Course**

**Neurology & Neuroradiology of Multiple Sclerosis: A Comprehensive Clinical Update**

27–28 November 2019
Cairo, Egypt

**Le 3ème Congrès Marocain de Neurophysiologie & La 4ème Session des Ecoles EEG & EMG**

29 November–1 December 2019
Marrakech, Morocco
Information: https://www.ilae.org/congresses/le-3-me-congr-s-marocain-de-neurophysiologie
American Epilepsy Society
6–10 December 2019
Baltimore, MD, USA
https://meeting.aesnet.org/abstracts

7th International Conference on Non-Invasive Brain Stimulation (NIBS)
24–26 March 2020
Baden-Baden/Germany
https://www.nibs-conference.de/

64. Jahrestagung
der Deutschen Gesellschaft für Klinische Neurophysiologie und Funktionele Bildgebung (64th annual meeting of the German Society of Clinical Neurophysiology)
26–28. March 2020
Baden-Baden, Germany
https://www.dgkn-kongress.de/

14th World Congress on Controversies in Neurology (CONy)
26–29 March 2020
London, UK
http://cony.comtecmed.com/

3rd International Training Course on Neuropsychology in Epilepsy
29 March–3 April 2020
Bordeaux, France
Information: https://www.ilae.org/congresses/3rd-international-training-course-on-neuropsychology-in-epilepsy

XI Congreso Latinamericano de Epilepsia
23–26 May 2020
Medellín, Colombia
Website: https://www.epilepsycongress.org/lace/

55. Jahrestagung der Deutschen Gesellschaft für Epileptologie (DGfE)
55th Annual Meeting of the German Society of Epileptology
10–13 June 2020

21st Annual Meeting of Infantile Seizure Society International Symposium on Pathophysiology of Developmental and Eileptic Encephalopathy (ISSET)
19–21 June 2020
Okayama, Japan

14th European Congress on Epileptology (ECE)
4–8 July 2020
Geneva Switzerland
Website: http://www.epilepsycongress.org/ece/

ESTM 2020: Epilepsy Surgery Techniques Meeting
9–10 July 2020
Geneva, Switzerland
https://www.estm2020.com/

2020 Advanced San Servolo Epilepsy Course
Bridging Basic with Clinical Epileptology - 7: Accelerating Translation in Epilepsy Research
20–31 July 2020
San Servolo (Venice), Italy
https://www.ilae.org/congresses/2020-advanced-san-servolo-epilepsy-course

First North American Epilepsy Congress (NAEC)
25–27 September 2020
Toronto, Canada

13th Asian and Oceanian Epilepsy Congress (AOEC)
8–11 October 2020
Fukuoka, Japan
https://www.epilepsycongress.org/congresses/aoec2020/