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Epilepsy Genetics: What Once Was Rare, Is Now Common

Ultrarare Genetic Variation in the Epilepsies: A Whole-Exome Sequencing Study of 17 606 Individuals

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Sequencing-based studies have identified novel risk genes associated with severe epilepsies and revealed an excess of rare deleterious variation in less-severe forms of epilepsy. To identify the shared and distinct ultrarare genetic risk factors for different types of epilepsies, we performed a whole-exome sequencing (WES) analysis of 9170 epilepsy-affected individuals and 8436 controls of European ancestry. We focused on 3 phenotypic groups: severe developmental and epileptic encephalopathies (DEEs), genetic generalized epilepsy (GGE), and nonacquired focal epilepsy (NAFE). We observed that compared to controls, individuals with any type of epilepsy carried an excess of ultrarare, deleterious variants in constrained genes and in genes previously associated with epilepsy; we saw the strongest enrichment in individuals with DEEs and the least strong in individuals with NAFE. Moreover, we found that inhibitory $GABA_A$ receptor genes were enriched for missense variants across all 3 classes of epilepsy, whereas no enrichment was seen in excitatory receptor genes. The larger gene groups for the GABAergic pathway or cation channels also showed a significant mutational burden in DEEs and GGE. Although no single gene surpassed exome-wide significance among individuals with GGE or NAFE, highly constrained genes and genes encoding ion channels were among the lead associations; such genes included CACNA1G, EEF1A2, and GABRG2 for GGE and LGI1, TRIM3, and GABRG2 for NAFE, respectively. Our study, the largest epilepsy WES study to date, confirms a convergence in the genetics of severe and less-severe epilepsies associated with ultrarare coding variation, and it highlights a ubiquitous role for GABAergic inhibition in epilepsy etiology.

Commentary

The majority of the epilepsies are now known to have a genetic basis, driven primarily by our unique ability to sequence large numbers of individuals with epilepsy. In particular, the early-onset and severe, developmental and epileptic encephalopathies (DEEs) can be attributed to a specific genetic cause in ~30% to 50% of cases.[1] Conversely, while heritability estimates suggest a significant genetic contribution, it has been challenging to pinpoint single genetic etiologies in the more common epilepsies, including genetic generalized epilepsy (GGE) and nonacquired focal epilepsy (NAFE). Moreover, it is currently unknown whether GGE and NAFE risk are primarily attributed to single gene variants of high risk (monogenic), or by multiple genetic variants with modest effect on risk (polygenic). This study, by the Epi25 collaborative, aimed to identify the rare genetic risk factors for epilepsy subtypes, primarily GGE and NAFE, in a large case–control exome study.[2]

The international collaborative performed exome sequencing analysis in 9170 individuals with epilepsy (GGE: 3108 | NAFE: 3597 | DEE: 1021 | other: 1444) and 8436 controls with matched genetic ancestry. Well-characterized pipelines were used to call single nucleotide variants and insertions and deletions (indels) and the emphasis was placed on ultrarare variants (URV), that is, variants present less than 4 times in the entire data set. These URVs are proposed to confer greater risk relative to more common variants of smaller effect. The authors perform burden analyses to test for enrichment of URVs exome-wide as well as for specific subsets of genes and single genes. Exome-wide significant enrichment for truncating URVs in highly constrained genes (probability of intolerance to heterozygous probable loss-of-function variation [pLI] > 0.995) was observed for DEE and GGE, though with modest effect sizes (odds ratios [ORs]) of 1.4 for both subtypes. Similarly, potentially damaging missense URVs in highly constrained genes were significantly enriched in GGE (OR = 1.1) and DEE (OR = 1.3). Nonacquired focal epilepsy showed no enrichment exome wide. These burden analyses in DEE are consistent with previous studies that show DEEs are caused by monogenic variants of large effect and revealed enrichment in known gene functions including GABAergic pathways and voltage-gated ion channels.

Subset gene burden analysis in the GGE cohort revealed significant enrichment for genes previously implicated in DEE and other neurodevelopmental disorders, as well as genes involved in GABAergic function. No single gene reached exome-wide significance and the best candidates for enrichment included *CACNA1G*. Interestingly, reduced *Cacna1g* expression modifies epilepsy phenotypes in 2 mouse sodium channelopathy models, supporting a link between *CACNA1G* variation and seizures.[3,4] Previous analysis of familial GGE revealed that URVs have moderate effect sizes (OR = 2.4) that are greater than sporadic GGE (OR = 1.1) as expected.[5] Similarly, no single gene in this familial data set reached exome-wide significance and there was no overlap for specific top candidate genes in familial versus sporadic GGE, though *CACNA1B*, another voltage-gated calcium channel, was the lead familial candidate, suggesting perhaps the same underlying pathway dysfunction. Collectively, these studies demonstrate a prevailing genetic contribution of URVs to familial and sporadic GGE, and further studies are needed to identify specific risk genes as well as the contribution of polygenic risk.

Nonacquired focal epilepsy was only enriched for predicted damaging missense URVs in a set of 43 epilepsy-associated genes as well as $GABA_A$ receptor genes. However, there was no enrichment exome-wide for sporadic NAFE, in contrast to previous findings in familial NAFE where effect sizes were even higher (OR = 3.6) than familial GEE (OR = 2.4).[5] Moreover, in a genome-wide association study, common variants accounted for greater heritability of generalized than focal epilepsy.[6] Collectively, this evidence suggests that the contribution of genetic risk may be higher in sporadic GGE than sporadic NAFE. However, one of the limitations of these case–control studies is the restriction to sequencing of DNA from (normally) blood rather than the affected organ (the brain). A number of studies have now shown that somatic mosaicism is emerging as a significant cause of NAFE, with examples including brain-specific *MTOR* variants in focal cortical dysplasia type 2 and *SLC35A2* variants in refractory nonlesional focal epilepsy.[7,8] It is an attractive hypothesis that the lack of strong genetic risk factors in sporadic NAFE in this study may be because genetic risk for NAFE is owing to somatic rather than germline genetic variation. In addition, NAFE may be more clinically, and thus also etiologically, more heterogeneous than GGE and also may extend to polygenic inheritance in some cases.

One of the primary limitations of this study, and indeed most genetic studies, is the inclusion of individuals with European Ancestry only. The authors estimate that in order to generate exome-wide significance for candidate GGE and NAFE genes, at least 8000 cases and 20 000 control samples will need to be sequenced. However, the greater diversity in non-Europeans, in particular in Africans provides greater power to detect disease-associated genes. For instance, a recent case–control analysis involving just 900 individuals with schizophrenia revealed that URVs in constrained genes were significantly enriched in individuals with schizophrenia with modest effect sizes (OR = 1.2).[9] These effect sizes are comparable to that seen in GGE (OR = 1.4), where 3 times more cases were sequenced, even though schizophrenia may be more heterogeneous than GGE. These results highlight the greater power to detect epilepsy-associated genes in more diverse populations. Moving forward, greater efforts and engagement are needed to involve African researchers in genetic studies in epilepsy, potentially within the H3Africa framework.[10] Finally, in this study, only 75% of the coding region was assessed in this analysis, as necessary stringency criteria filtered out ~25% of coding data. Sequencing to greater depth as well as the shift from exome sequencing to genome sequencing will also limit the problems capturing certain regions of the exome and increase power to detect disease associations.

Overall, this study demonstrates that GGE, and potentially NAFE, have an underlying genetic etiology that can be attributed, in part, to rare variants though larger studies and greater diversity are needed to identify single risk gene/genes and polygenic risk. As with the DEEs, this foundational shift will result in increasing generation of genetic models to study epilepsy and less of an emphasis on classic models such as kindling or status epilepticus models. Further, there is a much greater emphasis on a diversity of models, from zebrafish to mice to patient-derived neurons and cerebral organoids. The shared genetic etiology between DEE, GGE, and potentially NAFE means that the development of these models is useful for not only understanding the etiology of the rare epilepsies but also more common forms of epilepsy, thus providing a foundation for translational epilepsy research.

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References

1. Sanchez Fernandez I, Loddenkemper T, Gainza-Lein M, Sheidley BR, Poduri A. Diagnostic yield of genetic tests in epilepsy: a meta-analysis and cost-effectiveness study. *Neurology*. 2019:9.
2. Epi25 Collaborative. Ultra-rare genetic variation in the epilepsies: a whole-exome sequencing study of 17,606 individuals. *Am J Hum Genet*. 2019;105(2):267-282. doi:10.1016/j.ajhg.2019.05.020.
3. Calhoun JD, Hawkins NA, Zachwieja NJ, Kearney JA. Cacna1g is a genetic modifier of epilepsy caused by mutation of voltage-gated sodium channel Scn2a. *Epilepsia*. 2016;57(6):e103-e107.
4. Calhoun JD, Hawkins NA, Zachwieja NJ, Kearney JA. Cacna1 g is a genetic modifier of epilepsy in a mouse model of Dravet syndrome. *Epilepsia*. 2017;58(8):e111-e115.
5. Epi4K Consortium. Ultra-rare genetic variation in common epilepsies: a case-control sequencing study. *Lancet Neurol*. 2017;16(2):135-143.
6. 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(1):5269.

7. Lim JS, Kim WI, Kang HC, et al. Brain somatic mutations in MTOR cause focal cortical dysplasia type II leading to intractable epilepsy. *Nat Med*. 2015;21(4):395-400.
8. Winawer MR, Griffin NG, Samanamud J, et al. Somatic SLC35A2 variants in the brain are associated with intractable neocortical epilepsy. *Ann Neurol*. 2018;83(6):1133-1146.
9. Gulsuner S, Stein DJ, Susser ES, et al. Genetics of schizophrenia in the South African Xhosa. *Science*. 2020;367(6477): 569-573.
10. Rotimi C, Abayomi A, Abimiku A, et al. Enabling the genomic revolution in Africa. *Science*. 2014;344(6190):1346-1348.