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When Monogenic Isn't Monogenic—Unravelling the Oligogenic Architecture of the Developmental and Epileptic Encephalopathies

Comprehensive Analysis of Coding Variants Highlights Genetic Complexity in Developmental and Epileptic Encephalopathy

Takata A, Nakashima M, Saitsu H, et al. *Nat Commun*. 2019;10(1):2506. doi:10.1038/s41467-019-10482-9. www.nature.com/naturecommunications.

Although there are many known Mendelian genes linked to epileptic or developmental and epileptic encephalopathy (EE/DEE), its genetic architecture is not fully explained. Here, we address this incompleteness by analyzing exomes of 743 EE/DEE cases and 2366 controls. We observe that damaging ultra-rare variants (dURVs) unique to an individual are significantly over-represented in EE/DEE, both in known EE/DEE genes and the other non-EE/DEE genes. Importantly, enrichment of dURVs in non-EE/DEE genes is significant, even in the subset of cases with diagnostic dURVs ($P = 0.000215$), suggesting oligogenic contribution of non-EE/DEE gene dURVs. Gene-based analysis identifies exome-wide significant ($P = 2.04$ Å$\sim 10^{-6}$) enrichment of damaging de novo mutations in NF1, a gene primarily linked to neurofibromatosis, in infantile spasm. Together with accumulating evidence for roles of oligogenic or modifier variants in severe neurodevelopmental disorders, our results highlight genetic complexity in EE/DEE and indicate that EE/DEE is not an aggregate of simple Mendelian disorders.

Commentary

Our understanding of the etiologies of the developmental and epileptic encephalopathies (DEEs) has exploded in recent years.[1] Developmental and epileptic encephalopathies are the most severe group of epilepsies, typically beginning in early life. They are characterized by frequent uncontrolled seizures, often of multiple types, and developmental delay or intellectual disability. A key concept underpinning the term "epileptic encephalopathy" (EE) is the presence of frequent epileptiform activity that itself contributes to developmental slowing or regression.[2] The term was recently expanded to include "developmental" in recognition that many of the underlying etiologies of the DEEs result in developmental impairment in their own right, with the superimposed EE process further adversely impacting an individual's development.

Until the advent of gene discovery in the DEEs, these were considered to be acquired in origin. Next-generation sequencing now allows us to determine a causative pathogenic variant in around 50% of patients, with most having a de novo pathogenic variant. To date, the majority of pathogenic variants have been found in exons, which make up the 1% of the genome that encodes proteins. What about the remaining 50% of unsolved patients? Increasing interest in intronic regions harboring noncoding DNA is slowly revealing previously hidden mutations, such as poison exons[3] and repeat expansions,[4] that explain a proportion of these cases.

Yet the genetic architecture of the DEEs is likely to be far more complex than finding a single pathogenic variant might suggest. Clues to this complexity come from the broad spectrum of severity seen in most monogenic DEEs. For example, the prototypic genetic DEE, Dravet syndrome arises due to a *SCN1A* mutation in more than 80% of cases. Dravet syndrome has a broad phenotypic spectrum with half of the patients having severe intellectual disability, a third moderate and the remainder mild intellectual disability. Even patients with the same mutation may look surprisingly different. Dravet syndrome is associated with a range of comorbidities, many of which occur in only some cases, such as a crouch gait. What factors determine these differences in patients who share the same genetic etiology? Simple answers such as the nature or location of the *SCN1A* mutation have not answered these fundamental questions.[5]

An elegant statistical molecular study of a large DEE/EE cohort by Japanese authors has begun to reveal some tantalizing answers using a case–control approach. Takata and colleagues analyzed 3109 exomes, including 743 DEE/EE cases

and 2366 controls. They sequenced the 1% of the human genome called the exome that encodes proteins. In general, each human being has 22 000 variants in their exome, where his or her DNA nucleotide sequence differs from the general population. The challenge is to identify whether the variant is pathogenic, benign, or of unknown significance. The authors analyzed both common and rare variants, with a focus on ultra-rare variants (URVs), which they defined as only occurring once in their cohort and not found in population databases such as the Exome Aggregation Consortium, gnomAD, and the Tohoku Medical Megabank Organisation. They identified a total of 169 014 and 42 974 variants in coding and noncoding regions, respectively. They acknowledge that their restricted analysis based on their definition of URVs would miss recurrent pathogenic variants in their cohort or the general population, and disease-contributing variants in the general population; however, their approach allowed an unbiased analysis to detect enrichment of rare deleterious variants.

Overall, they found an excess of damaging URVs (dURVs) in patients with DEE/EEs compared to controls, both in known DEE/EE genes and in non-DEE/EE genes. Damaging ultra-rare variants were defined as either null (nonsense, frameshift, splice site, and read-through; resulting in loss-of-function) or consensus-damaging missense variants, based on their prediction as damaging by multiple in silico prediction algorithms. Although dURVs in DEE/EE genes were 10 times more frequent in cases than controls, 39 dURVs were identified in controls emphasizing the importance of careful genotype–phenotype analysis in all cases. Equally, the finding of dURVs in the 18 338 non-58 known DEE/EE genes highlights potential novel genes for DEEs. Somewhat intriguingly, the enrichment of dURVs in non-DEE/EE genes was also observed in the 116 cases with a known causative pathogenic variant in one of the 58 known DEE/EE genes. Their gene expression data analysis of various tissues and cell types supported the role of the non-DEE/EE gene in brain development and function.

Unsurprisingly, they found pathogenic URVs in non-DEE/EE genes that have been reported in other neurodevelopmental phenotypes such as autism spectrum disorder, reinforcing the known overlap in the genetic basis of neurodevelopmental disorders.[6] They suggest these may contribute to the oligogenic architecture of a patient's disorder, particularly in individuals who do not have a pathogenic URV in a known DEE/EE gene.

They performed gene-based burden testing and found an exome-wide significant burden of dURVs in well-known DEE/EE genes, *CDKL5, STXBP1, SCN1A, SCN2A*, and *KCNQ2*. They also found overlap with genes identified in the common epilepsies studied by EPI4K, particularly the genetic generalized epilepsies.[7]

No single gene in the non-DEE/EE genes reached exome-wide significance; however, the most enriched gene was *NF1*, known to cause neurofibromatosis, followed by *TRPM5, AP5B1, DNMT3L*, and *ARFGEF1*. They pursued the 3 cases with dURVs in *NF1*; all had arisen de novo and each patient had infantile spasms, 2 with the stigmata of neurofibromatosis. When analysed separately, *NF1* surpassed the exome-wide significance threshold in the subset of probands with infantile spasms drawn from their overall DEE cohort.

They interrogated their data set for a common exonic single nucleotide polymorphism (SNP), defined as having a minor allele frequency of greater than 1%, without success. They rightly point out that their sample size, albeit reasonably large, is probably not sufficient to identify common exonic SNPs of small effect or low-frequency SNPs of intermediate effect. This will require larger cohorts, especially if we are to focus on specific epilepsy syndromes. Sample size remains a critical issue for all aspects of cohort studies exploring the genetic architecture of common diseases such as epilepsy. International consortia, such as EPI25, are bringing together larger patient groups.[8] Their recent study of exome sequencing in 9170 patients with epilepsy showed that there was an excess of ultra-rare deleterious variation in constrained genes, and known epilepsy genes, and a convergence of implicated genes across the DEEs, genetic generalized epilepsies and nonacquired focal epilepsies. Although some of the findings reflected those of Takata and colleagues, the EPI25 study highlights the need for even larger data sets to unravel the complex genetic architecture of the epilepsies.

So what does this mean? These findings suggest that these patients actually have at least an oligogenic architecture for their DEEs, rather than being simple Mendelian disorders. Equally though, these additional dURVs may not result in disease without the accompanying pathogenic variant in a gene of major effect. Nevertheless, it is likely that some patients with DEEs will have a polygenic basis. To date, this has been too complex to unravel but, in the future, it is likely that we will be able to drill down on those patients who may have a DEE due to 10 or even tens of genetic variants, each of low effect, but contributing to a similarly devastating disease. In considering the full gamut of etiologies, the impact of environmental factors on an oligogenic or polygenic background is a challenge yet to be tackled.

These additional variants may be considered modifier genes likely influencing the phenotypic picture, treatment responses, and prognosis. How do the additional dURVs help us in the clinic? Probably not today, but in the not-too-distant future, they may inform about efficacy and tolerability of specific antiepileptic drugs, the risk and severity of comorbidities, risk of SUDEP, and long-term outcome.

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