Targeted Resequencing in Epileptic Encephalopathies Identifies De Novo Mutations in CHD2 and SYNGAP1.


Epileptic encephalopathies are a devastating group of epilepsies with poor prognosis, of which the majority are of unknown etiology. We perform targeted massively parallel resequencing of 19 known and 46 candidate genes for epileptic encephalopathy in 500 affected individuals (cases) to identify new genes involved and to investigate the phenotypic spectrum associated with mutations in known genes. Overall, we identified pathogenic mutations in 10% of our cohort. Six of the 46 candidate genes had 1 or more pathogenic variants, collectively accounting for 3% of our cohort. We show that de novo CHD2 and SYNGAP1 mutations are new causes of epileptic encephalopathies, accounting for 1.2% and 1% of cases, respectively. We also expand the phenotypic spectra explained by SCN1A, SCN2A and SCN8A mutations. To our knowledge, this is the largest cohort of cases with epileptic encephalopathies to undergo targeted resequencing. Implementation of this rapid and efficient method will change diagnosis and understanding of the molecular etiologies of these disorders.

Exome Sequencing Reveals New Causal Mutations in Children With Epileptic Encephalopathies.


PURPOSE: The management of epilepsy in children is particularly challenging when seizures are resistant to antiepileptic medications, or undergo many changes in seizure type over time, or have comorbid cognitive, behavioral, or motor deficits. Despite efforts to classify such epilepsies based on clinical and electroencephalographic criteria, many children never receive a definitive etiologic diagnosis. Whole exome sequencing (WES) is proving to be a highly effective method for identifying de novo variants that cause neurologic disorders, especially those associated with abnormal brain development. Herein we explore the utility of WES for identifying candidate causal de novo variants in a cohort of children with heterogeneous sporadic epilepsies without etiologic diagnoses. METHODS: We performed WES (mean coverage approximately 40×) on 10 trios comprised of unaffected parents and a child with sporadic epilepsy characterized by difficult-to-control seizures and some combination of developmental delay, epileptic encephalopathy, autistic features, cognitive impairment, or motor deficits. Sequence processing and variant calling were performed using standard bioinformatics tools. A custom filtering system was used to prioritize de novo variants of possible functional significance for validation by Sanger sequencing. KEY FINDINGS: In 9 of 10 probands, we identified one or more de novo variants predicted to alter protein function, for a total of 15. Four probands had de novo mutations in genes previously shown to harbor heterozygous mutations in patients with severe, early onset epilepsies (two in SCN1A, and one each in CDKL5 and EEF1A2). In three children, the de novo variants were in genes with functional roles that are plausibly relevant to epilepsy (KCNH5, CLCN4, and ARHGEF15). The variant in KCNH5 alters one of the highly conserved arginine residues of the voltage sensor of the encoded voltage-gated potassium channel. In vitro analyses using cell-based assays revealed that the CLCN4 mutation greatly impaired ion transport by the ClC-4 2Cl−/H+-exchanger and that the mutation in ARHGEF15 reduced GEF exchange activity of the gene product, Ephexin5, by about 50%. Of interest, these seven probands all presented with seizures within the first 6 months of life, and six of these have intractable seizures. SIGNIFICANCE: The finding that 7 of 10 children carried de novo mutations in genes of known or plausible clinical significance to neuronal excitability suggests that WES will be of use for the molecular genetic diagnosis of sporadic epilepsies in children, especially when seizures are of early onset and difficult to control.
Commentary
Epileptic encephalopathies are a heterogeneous group of catastrophic epilepsies characterized by treatment-resistant seizures, behavioral and neurocognitive deficits, and a poor prognosis. Until recently, the causes of epileptic encephalopathies were largely unknown; however, emerging data from recent studies indicate that genetic factors are likely to account for a substantial percentage of cases (1–7).

Two recent studies that are the focus of this commentary provide further support for the genetic basis of epileptic encephalopathies. These studies used different but complementary genetic screening methods, namely, whole-exome sequencing (WES) and targeted resequencing. WES is an unbiased approach to disease gene discovery in which the protein coding regions of almost all the genes in the genome are sequenced and then compared with reference sequences. Targeted resequencing typically involves the application of high-throughput methods to perform comprehensive sequence analysis on pre-selected known or candidate disease genes in large numbers of samples.

Veeramah et al. (2013) performed WES on ten trios, each consisting of an affected child and both unaffected parents. Inclusion of the parents provided an efficient strategy to identify potentially disease-causing de novo mutations that, by definition, would only be present in the child. The ten children all shared histories of treatment-resistant epilepsy, but their seizure types and associated comorbid features differed. Of importance, despite the small number of patients and their range of clinical presentations, de novo mutations in known epilepsy genes or plausible candidate genes were identified in seven probands. Four probands had mutations in three genes previously associated with severe epilepsy: the voltage-gated sodium channel SCN1A, cyclin-dependent kinase-like 5 (CDKL5), and EEF1A2, encoding the eukaryotic translation factor 1 alpha 2. New candidate epilepsy genes that emerged from this study included a voltage-gated potassium channel (KCNH5), a voltage-dependent 2Cl⁻/H⁺ exchanger (CLCN4), and the Rho guanine nucleotide exchange factor 15 (ARHGEF15 or Ephexin5). Not surprisingly, several variants of unclear functional significance were also identified.

Carvill et al. (2013) performed high-throughput targeted sequencing of 19 known and 46 candidate epilepsy genes in 500 individuals with a range of epileptic encephalopathy phenotypes. The authors identified pathogenic or likely pathogenic mutations in 10% of the patients. Sixteen genes were found to harbor potentially disease-causing mutations. Of interest, multiple patients had mutations in CHD2, which encodes a member of the chromodomain helicase DNA-binding family of proteins, and SYNGAP1, a RAS/RAF GTP-activating protein. These genes, not previously implicated in epileptic encephalopathies, each accounted for approximately 1% of cases and are therefore likely to be important causal genes. Furthermore, the six patients with CHD2 mutations shared distinctive clinical features, raising the possibility that a better understanding of the underlying genetic architecture of epileptic encephalopathies might eventually help uncover discrete clinical subtypes. In addition, several genes that were previously associated with other epilepsy disorders, for example, the SCN2A voltage-gated sodium channel, were found to also significantly contribute to epileptic encephalopathies.

Taken together, the findings from these two studies, along with several other recent reports (1–7), have already established that genetic factors (gene mutations as well as pathogenic copy number variants) are responsible for at least 20% of epileptic encephalopathies. Clearly, as more studies are conducted, the contribution of genetics to this class of epilepsies will continue to increase. The observation that a large percentage of identified causal variants are de novo mutations is significant, since in a clinical setting, an underlying genetic cause would typically only be suspected in patients with a family history of epilepsy. However, the significant contribution of de novo mutations to the epileptic encephalopathies suggests that genetic analysis should be considered in all cases of unknown etiology. The routine application of genetics-based diagnoses is likely to become more feasible as advances in sequencing technologies lead to lower costs. Nevertheless, several challenges remain. Comprehensive sequence analysis of each DNA sample also results in the identification of large numbers of nonpathogenic genetic variants. Recognition of the disease-causing mutation against this background of genetic variation is akin to finding the proverbial needle in the haystack. While improvements in bioinformatic algorithms and strategies will continue to improve the efficiency of analyzing sequence data, at the present time, comprehensive sequence analysis is primarily only feasible in research settings. Furthermore, as illustrated by these two studies, sequence analysis reveals large numbers of variants of unknown significance, as well as putative disease genes with unclear roles in epilepsy. Extensive basic research efforts will therefore be required to definitively establish disease-causing roles for many identified variants. Eventually, more comprehensive panels of epileptic encephalopathy genes will be developed, thereby facilitating cost-effective and rapid identification of causal mutations. Genetics-based diagnosis of epileptic encephalopathies holds the promise for the identification of more discrete epilepsy subtypes, and ultimately the selection of more gene-specific treatment strategies.

by Andrew Escayg, PhD, and Jennifer C. Wong, PhD

References


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