EPILEPSY CURRENTS

Current Literature

In Basic Science

Epi4K Phase I: Gene Discovery in Epileptic Encephalopathies by Exome Sequencing

***De Novo* Mutations in Epileptic Encephalopathies.**

Epi4K Consortium; Epilepsy Phenome/Genome Project, Allen AS, Berkovic SF, Cossette P, Delanty N, Dlugos D, Eichler EE, Epstein MP, Glauser T, Goldstein DB, Han Y, Heinzen EL, Hitomi Y, Howell KB, Johnson MR, Kuzniecky R, Lowenstein DH, Lu YF, Madou MR, Marson AG, Mefford HC, Esmaeeli Nieh S, O'Brien TJ, Ottman R, Petrovski S, Poduri A, Ruzzo EK, Scheffer IE, Sherr EH, Yuskaitis CJ, Abou-Khalil B, Alldredge BK, Bautista JF, Berkovic SF, Boro A, Cascino GD, Consalvo D, Crumrine P, Devinsky O, Dlugos D, Epstein MP, Fiol M, Fountain NB, French J, Friedman D, Geller EB, Glauser T, Glynn S, Haut SR, Hayward J, Helmers SL, Joshi S, Kanner A, Kirsch HE, Knowlton RC, Kossoff EH, Kuperman R, Kuzniecky R, Lowenstein DH, McGuire SM, Motika PV, Novotny EJ, Ottman R, Paolicchi JM, Parent JM, Park K, Poduri A, Scheffer IE, Shellhaas RA, Sherr EH, Shih JJ, Singh R, Sirven J, Smith MC, Sullivan J, Lin Thio L, Venkat A, Vining EP, Von Allmen GK, Weisenberg JL, Widdess-Walsh P, Winawer MR. *Nature* 2013;501:217–221.

Epileptic encephalopathies are a devastating group of severe childhood epilepsy disorders for which the cause is often unknown. Here we report a screen for *de novo* mutations in patients with two classical epileptic encephalopathies: infantile spasms ($n = 149$) and Lennox–Gastaut syndrome ($n = 115$). We sequenced the exomes of 264 probands, and their parents, and confirmed 329 *de novo* mutations. A likelihood analysis showed a significant excess of *de novo* mutations in the ~4,000 genes that are the most intolerant to functional genetic variation in the human population ($P = 2.9 \times 10^{-3}$). Among these are *GABRB3*, with *de novo* mutations in four patients, and *ALG13*, with the same *de novo* mutation in two patients; both genes show clear statistical evidence of association with epileptic encephalopathy. Given the relevant site-specific mutation rates, the probabilities of these outcomes occurring by chance are $P = 4.1 \times 10^{-10}$ and $P = 7.8 \times 10^{-12}$, respectively. Other genes with *de novo* mutations in this cohort include *CACNA1A*, *CHD2*, *FLNA*, *GABRA1*, *GRIN1*, *GRIN2B*, *HNRNPU*, *IQSEC2*, *MTOR* and *NEDD4L*. Finally, we show that the *de novo* mutations observed are enriched in specific gene sets including genes regulated by the fragile X protein ($P < 10^{-8}$), as has been reported previously for autism spectrum disorders.

Commentary

The Epi4K Consortium was launched in 2011 in response to a National Institute of Neurological Disorders and Stroke (NINDS) Funding Opportunity Announcement soliciting applications for "Centers Without Walls for Collaborative Research in the Epilepsies: Genetics and Genomics of Human Epilepsies." This large-scale collaborative effort leverages large sample cohorts to increase statistical power and accelerate epilepsy gene discovery. The ultimate goal of the Epi4K Center without Walls is to sequence the genomes of at least 4,000 individuals with epilepsy using next-generation sequencing (NGS) (1).

NGS technology has revolutionized our ability to efficiently and inexpensively sequence individuals at the whole exome or whole genome level. The cost of whole exome sequencing is now under $1000 per sample. Whole exome sequencing studies have been quite successful for identifying *de novo* dominant mutations in individuals with profound neurodevelopmental phenotypes (2, 3). This approach relies on a simple genetic model in which the causative mutation is assumed to be present in the genome of the affected child but absent in the unaffected parents. On average, each individual carries a mutation in one of their genes that was not present in their parents (4) and, thus, is denoted as a *de novo* mutation. The occurrence of *de novo* mutations in the same gene in two or more unrelated individuals with similar clinical phenotypes supports a potential pathogenic contribution to the disease.

The first project tackled by the Epi4K Consortium focused on discovery of *de novo* mutations in two well-defined epileptic encephalopathies: Lennox–Gastaut syndrome and infantile spasms. Patients were ascertained by the NINDS-funded Epilepsy Phenome/Genome Project, a multi-center collaborative effort designed to collect detailed, high-quality phenotype information for genetic studies (5). The Epi4K Consortium performed whole exome sequencing on 264 patient–parent trios, of which 149 were classified as infantile spasms and 115 were Lennox–Gastaut syndrome. They identified 329 confirmed *de novo* mutations. On average, each patient harbored 1.25 de novo mutations, which is consistent with other studies reported in the literature (4, 6). The majority of the mutations

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(72%) were missense (encoding an amino acid change), while 7.5% resulted in premature stop codons. Relative to control populations, the observed rate of loss-of-function mutations was significantly elevated in the patients.

Nine genes were found to have *de novo* mutations in two or more probands, of which five genes had an already known association with epileptic encephalopathy. The known epileptic encephalopathy genes with mutations in multiple Epi4K subjects were: *SCN1A*, *STXBP1*, *SCN8A*, *SCN2A*, and *CDKL5*. Additional novel genes with mutations in multiple individuals included*: GABRB3, ALG13, CACNA1A, CHD2, FLNA, GABRA1, GRIN1, GRIN2B, HNRNPU, IQSEC2, MTOR*, and *NEDD4L*. To evaluate the novel genes, the authors applied a more rigorous standard that takes into account the local mutation rate, gene size, and mutation intolerance. Using this approach, they found statistical evidence of association between epileptic encephalopathy and *GABRB3* and *ALG13*.

De novo missense mutations in *GABRB3* were observed in four individuals, including one with infantile spasms only and three with infantile spasms that evolved to Lennox–Gastaut syndrome. Although missense mutations in *GABRB3* had previously been associated with childhood absence epilepsy (7), this report expands the phenotype range of this gene to include epileptic encephalopathies. Future functional studies will be required to determine the underlying molecular mechanisms and understand how mutations in *GABRB3* lead to seemingly disparate clinical syndromes.

ALG13 is an X-linked gene that encodes a subunit of the uridine diphosphate-N-acetylglucosamine transferase. *De novo* mutations in *ALG13* had previously been associated with severe intellectual disability with seizures and dysmorphic features (8), and a glycosylation disorder with microcephaly, seizures, and early lethality (9). In the current study, the same missense mutation was observed in two unrelated patients, one with infantile spasms only and one with infantile spasms that evolved to Lennox–Gastaut syndrome. Interestingly, this mutation was identical to the mutation previously reported by de Ligt and colleagues in a patient with intellectual disability, seizures, and dysmorphic features (8). Together, these reports indicate that there is considerable phenotype heterogeneity associated with mutations in *ALG13*. Again, functional studies will be necessary to understand the mechanisms by which ALG13 mutations result in variable developmental disorders.

The collective list of epileptic encephalopathy genes reported by Epi4K has substantial overlap with genes identified in studies of other neurodevelopmental disorders, including intellectual disability and autism (2, 3, 6). This high degree of phenotype heterogeneity suggests that other factors, including environmental, stochastic, and genetic background effects, interact with the mutation during development to shape the distinct clinical phenotype that is expressed in an individual. Epileptic encephalopathies also exhibit considerable locus heterogeneity, with mutations in many different genes resulting in infantile spasms or Lennox–Gastaut syndrome. Based on the observed locus heterogeneity, there are likely more epileptic encephalopathy genes in the long list of single-hit genes reported by the Epi4K Consortium in a supplementary table. A protein–protein interaction analysis on all genes that had at least one *de novo* mutation revealed a network of 71 interconnected proteins, including six previously associated with epileptic encephalopathy. Approximately 20% of the single-hit genes fit in this network, strongly suggesting that they may represent additional epileptic encephalopathy genes. Follow-up in larger patient cohorts will likely uncover additional mutations in some of these genes and validate them as epileptic encephalopathy genes. Additionally, future functional studies will provide further support and advance our understanding of the underlying mechanisms.

The high degree of locus heterogeneity observed in epileptic encephalopathies has practical implications for molecular diagnostics. It highlights the need for a strategy shift away from gene-by-gene or gene panel screening. Given that it is difficult to predict the gene likely to be mutated, clinical exome sequencing is the most expeditious and cost–effective path to genetic diagnosis once proximate causes have been ruled out and a genetic basis is likely. There are challenges for integrating exome sequencing into clinical practice, including interpretation and counseling, diagnostic yield and insurance authorization. However, similar obstacles existed for earlier generations of genetic testing, like single gene tests, and they were overcome with education, accumulating knowledge, and shifting norms. Ultimately, rapid genetic diagnosis is another tool that will improve disease management and may lead to more favorable long-term outcomes. Moreover, genetic diagnosis is valuable to the family for establishing clinical and social support services, family planning, and alleviating the stress of diagnostic uncertainty.

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