“Idiopathic” and “Genetic” Generalized Epilepsies Intersect

Exome Sequencing Followed by Large-Scale Genotyping Fails to Identify Single Rare Variants of Large Effect in Idiopathic Generalized Epilepsy.

Heinzen EL, Depondt C, Cavalleri GL, Ruzzo EK, Walley NM, Need AC, Ge D, He M, Cirulli ET, Zhao Q, Cronin KD, Gumbs CE, Campbell CR, Hong LK, Maia JM, Shianna KV, McCormack M, Radtke RA, O’Conner GD, Mikati MA, Gallentine WB, Husain AM, Sinha SR, Chinthapalli K, Puranam RS, McNamara JO, Ottman R, Sisodiya SM, Delanty N, Goldstein DB. *Am J Hum Genet* 2012;91:293–302.

Idiopathic generalized epilepsy (IGE) is a complex disease with high heritability, but little is known about its genetic architecture. Rare copy-number variants have been found to explain nearly 3% of individuals with IGE; however, it remains unclear whether variants with moderate effect size and frequencies below what are reliably detected with genome-wide association studies contribute significantly to disease risk. In this study, we compare the exome sequences of 118 individuals with IGE and 242 controls of European ancestry by using next-generation sequencing. The exome-sequenced epilepsy cases include study subjects with two forms of IGE, including juvenile myoclonic epilepsy ($n = 93$) and absence epilepsy ($n = 25$). However, our discovery strategy did not assume common genetic control between the subtypes of IGE considered. In the sequence data, as expected, no variants were significantly associated with the IGE phenotype or more specific IGE diagnoses. We then selected 3,897 candidate epilepsy-susceptibility variants from the sequence data and genotyped them in a larger set of 878 individuals with IGE and 1,830 controls. Again, no variant achieved statistical significance. However, 1,935 variants were observed exclusively in cases either as heterozygous or homozygous genotypes. It is likely that this set of variants includes real risk factors. The lack of significant association evidence of single variants with disease in this two-stage approach emphasizes the high genetic heterogeneity of epilepsy disorders, suggests that the impact of any individual single-nucleotide variant in this disease is small, and indicates that gene-based approaches might be more successful for future sequencing studies of epilepsy predisposition.

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, Esmaeili 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.

Copy Number Variants are Frequent in Genetic Generalized Epilepsy with Intellectual Disability.

Mullen SA, Carvill GL, Bellows S, Bayly MA, Berkovic SF, Dibbens LM, Scheffer IE, Mefford HC. *Neurology* 2013;81:1507–1514.

OBJECTIVE: We examined whether copy number variants (CNVs) were more common in those with a combination of intellectual disability (ID) and genetic generalized epilepsy (GGE) than in those with either phenotype alone via a case-control study. **METHODS:** CNVs contribute to the genetics of multiple neurodevelopmental disorders with complex inheritance, including GGE and ID. Three hundred fifty-nine probands with GGE and 60 probands with ID-GGE were screened for GGE-associated recurrent microdeletions at 15q13.3, 15q11.2, and 16p13.11 via quantitative PCR or loss of heterozygosity. Deletions were confirmed by comparative genomic hybridization (CGH). ID-GGE probands also had genome-wide CGH. **RESULTS:** ID-GGE probands showed a significantly higher rate of CNVs compared with probands with GGE alone, with 17 of 60 (28%) ID-GGE probands having one or more potentially causative CNVs. The patients with ID-GGE had a 3-fold-higher rate of the 3 GGE-associated recurrent microdeletions than probands with GGE alone (10% vs 3%, $p = 0.02$). They also showed a high rate (13/60, 22%) of rare CNVs identified using genome-wide CGH. **CONCLUSIONS:** This study shows that CNVs are common in those with ID-GGE with recurrent deletions at 15q13.3, 15q11.2, and 16p13.11, particularly enriched compared with individuals with GGE or ID alone. Recurrent CNVs are likely to act as risk factors for multiple phenotypes not just at the population level, but also in any given individual. Testing for CNVs in ID-GGE will have a high diagnostic yield in a clinical setting and will inform genetic counseling.

Epilepsy, Hippocampal Sclerosis and Febrile Seizures Linked by Common Genetic Variation Around *SCN1A*.

Kasperavičiute D, Catarino CB, Matarin M, Leu C, Novy J, Tostevin A, Leal B, Hessel EV, Hallmann K, Hildebrand MS, Dahl HH, Ryten M, Trabzuni D, Ramasamy A, Alhusaini S, Doherty CP, Dorn T, Hansen J, Krämer G, Steinhoff BJ, Zumsteg D, Duncan S, Kälviäinen RK, Eriksson KJ, Kantanen AM, Pandolfo M, Gruber-Sedlmayr U, Schlachter K, Reinthaler EM, Stogmann E, Zimprich F, Théâtre E, Smith C, O'Brien TJ, Meng Tan K, Petrovski S, Robbiano A, Paravidino R, Zara F, Striano P, Sperling MR, Buono RJ, Hakonarson H, Chaves J, Costa PP, Silva BM, da Silva AM, de Graan PN, Koeleman BP, Becker A, Schoch S, von Lehe M, Reif PS, Rosenow F, Becker F, Weber Y, Lerche H, Rössler K, Buchfelder M, Hamer HM, Kobow K, Coras R, Blumcke I, Scheffer IE, Berkovic SF, Weale ME; UK Brain Expression Consortium, Delanty N, Depondt C, Cavalleri GL, Kunz WS, Sisodiya SM. *Brain* 2013;136:3140–3150.

Epilepsy comprises several syndromes, amongst the most common being mesial temporal lobe epilepsy with hippocampal sclerosis. Seizures in mesial temporal lobe epilepsy with hippocampal sclerosis are typically drug-resistant, and mesial temporal lobe epilepsy with hippocampal sclerosis is frequently associated with important co-morbidities, mandating the search for better understanding and treatment. The cause of mesial temporal lobe epilepsy with hippocampal sclerosis is unknown, but there is an association with childhood febrile seizures. Several rarer epilepsies featuring febrile seizures are caused by mutations in *SCN1A*, which encodes a brain-expressed sodium channel subunit targeted by many anti-epileptic drugs. We undertook a genome-wide association study in 1018 people with mesial temporal lobe epilepsy with hippocampal sclerosis and 7552 control subjects, with validation in an independent sample set comprising 959 people with mesial temporal lobe epilepsy with hippocampal sclerosis and 3591 control subjects. To dissect out variants related to a history of febrile seizures, we tested cases with mesial temporal lobe epilepsy with hippocampal sclerosis with (overall $n = 757$) and without (overall $n = 803$) a history of febrile seizures. Meta-analysis revealed a genome-wide significant association for mesial temporal lobe epilepsy with hippocampal sclerosis with febrile seizures at the sodium channel gene cluster on chromosome 2q24.3 [rs7587026, within an intron of the *SCN1A* gene, $P = 3.36 \times 10^{-9}$, odds ratio (A) = 1.42, 95% confidence interval: 1.26–1.59]. In a cohort of 172 individuals with febrile seizures, who did not develop epilepsy during prospective follow-up to age 13 years, and 6456 controls, no association was found for rs7587026 and febrile seizures. These findings suggest *SCN1A* involvement in a common epilepsy syndrome, give new direction to biological understanding of mesial temporal lobe epilepsy with hippocampal sclerosis with febrile seizures, and open avenues for investigation of prognostic factors and possible prevention of epilepsy in some children with febrile seizures.

Commentary

Several recent genomic sequencing studies may “shake up” our view of the generalized epilepsies. The idiopathic generalized syndromes (IGE)—childhood and juvenile absence epilepsy and juvenile myoclonic epilepsies—appear to only rarely originate from de novo or inherited mutations of single

genes. Rather, IGE may be something more like height, or intelligence, or athletic ability—a human phenotype resulting from polygenic inheritance, representing the interaction between multiple genes and the environment. This “bell curve” of cortical excitability may result in some patients expressing a syndrome of seizures beginning in childhood and adolescence,

while family members may have cortical increased excitability as shown recently with transcranial magnetic stimulation, but without seizures. This group of patients with IGE, however, appears to overlap with a second distribution of patients with generalized seizures and mild to severe intellectual disability. Analogous to extreme short stature due to achondroplasia or intellectual disability caused by untreated PKU, these patients often have specific genetic disorders, such as copy number variants or single gene mutations. These recent studies of IGE and the "genetic" generalized epilepsies point to an exciting new way to reframe our understanding about epilepsy.

In an article descriptively titled "Exome Sequencing Followed by Large-Scale Genotyping Fails to Identify Single Rare Variants of Large Effect in Idiopathic Generalized Epilepsy," Heinzen et al. showed that 118 patients with Juvenile Myoclonic Epilepsy (JME) and Absence Epilepsy (AE) did not share major gene defects. The study illustrated the daunting informatics involved in genomic screening: Patients had 87,255 high-quality functional variants that had an estimated population frequency of less than 5 percent. Patients in both the main study cohort and in a follow-up cohort of patients with IGE did not share common variants. This negative screening for single shared gene mutations supports the emerging idea that cortical excitability in IGE is a complex polygenic trait.

Some patients' epilepsies, however, are clearly caused by single gene mutations, which can be firmly placed in the category of generalized genetic epilepsies. The Epi4K Consortium and Epilepsy Phenome/Genome Project, in a study typical of several recent large national and multi-national screening projects, recently reported 329 de novo mutations among 264 patients with infantile spasms or Lennox Gastaut Syndrome. Rather than classical Mendelian inheritance, these patients show the importance of de novo autosomal dominant mutations in causing disease. Most of these de novo changes were found in only a single patient and so cannot necessarily be firmly linked to their disorder. Some patients had mutations previously tied to an epileptic encephalopathy—*SCN1A*, *STXBP1*, *SCN8A*, *SCN2A*, and *CDKL5*—as well as some epilepsy-linked genes, such as *GABRB3* and *ALG13*, which were not previously firmly implicated in epileptic encephalopathies. Using polymorphism data, they concluded that up to 90 change-intolerant genes may contribute to the epileptic encephalopathies in an interconnected web of protein-protein interactions.

A fascinating third gene sequencing study by Mullen et al. reports an intersection between genetic studies of IGE and the epileptic encephalopathies. The authors speculated that larger proportions of patients with intellectual disability and "idio-

pathic generalized epilepsy" would have CNVs compared to patients with IGE and normal intellect. The majority of patients (37/60) had borderline intellect (defined as an IQ of 70 to 85). They found 28 percent of the patients with intellectual disability and generalized epilepsy had a potentially causative CNV. Ten percent of the patients had microdeletions in gene regions previously associated with generalized epilepsy (15q13.3, 15q11.2, and 16p13.11). Patients with IGE and normal intellect had fewer rare CNVs, and only 3 percent had these specific microdeletions. A large proportion of patients with atypical "early onset" IGE (between 1 and 4 years) had CNVs (7/19) compared to patients with onset >4 years of age (15/400); all these "early onset" patients had intellectual disability. This demonstrates that genetic abnormalities associated with intellectual disability and seizures are common in early onset IGE.

These genetic findings raise questions regarding appropriate nomenclature for the generalized epilepsies. Perhaps "genetic generalized epilepsy" should be a diagnosis reserved for patients with known CNVs or single gene mutations, since variants with low penetrance may have complex gene expression which may be influenced by environmental factors. An example is a recently described link between epilepsy, hippocampal sclerosis and febrile seizures, and genetic variation around *SCN1A* by Kasperaviciute et al. Those patients with generalized epilepsy associated with early age of onset, borderline intelligence or intellectual disability, or atypical syndromic patterns such as treatment-resistant absences might be better designated as having "symptomatic generalized epilepsy," thus indicating that further genetic evaluation might be warranted to better understand and potentially treat their seizures.

An exciting aspect of these findings is that both the increased cortical excitability associated with complex polygenic inheritance in IGE and traits linked with many of the CNVs and single gene mutations (e.g., schizophrenia, autism spectrum disorder, intellectual disability, infantile spasms) may illustrate secondary regulatory targets for treating these disorders. The web of pathways involved in these disorders point to potential rodent models for the two spectrums of disorders: polygenic models of increased cortical excitability that screen for inhibitory modulators treating IGE and more specific "end of pathway" models of the epileptic encephalopathies and related disorders which might screen therapies to treat large numbers of de novo dominant mutations that appear to interfere with these pathways.

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