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Solving the Molecular Basis of the Developmental and Epileptic Encephalopathies: Are We there Yet?

Keywords
NGS, adults, gene panel, genetic testing

Diagnostic Yield of Whole Genome Sequencing After Nondiagnostic Exome Sequencing or Gene Panel in Developmental and Epileptic Encephalopathies

Palmer EE, Sachdev R, Macintosh R, et al. *Neurology*. 2021;96(13):e1770-e1782. doi:10.1212/WNL.0000000000011655

Objective: To assess the benefits and limitations of whole genome sequencing (WGS) compared to exome sequencing (ES) or multigene panel (MGP) in the molecular diagnosis of developmental and epileptic encephalopathies (DEEs). Methods: We performed WGS of 30 comprehensively phenotyped DEE patient trios that were undiagnosed after first-tier testing, including chromosomal microarray and either research ES (n = 15) or diagnostic MGP (n = 15). Results: Eight diagnoses were made in the 15 individuals who received prior ES (53%): 3 individuals had complex structural variants; 5 had ES-detectable variants, which now had additional evidence for pathogenicity. Eleven diagnoses were made in the 15 MGP-negative individuals (68%); the majority (n = 10) involved genes not included in the panel, particularly in individuals with postneonatal onset of seizures and those with more complex presentations including movement disorders, dysmorphic features, or multiorgan involvement. A total of 42% of diagnoses were autosomal recessive or X-chromosome linked. Conclusion: WGS was able to improve diagnostic yield over ES primarily through the detection of complex structural variants (n = 3). The higher diagnostic yield was otherwise better attributed to the power of re-analysis rather than inherent advantages of the WGS platform. Additional research is required to assist in the assessment of pathogenicity of novel noncoding and complex structural variants and further improve diagnostic yield for patients with DEE and other neurogenetic disorders.

Utility of Genetic Testing for Therapeutic Decision-Making in Adults with Epilepsy

Johannesen KM, Nikanorova N, Marjanovic D, et al. *Epilepsia*. 2020;61(6):1234-1239, 2020. doi: 10.1111/epi.16533
Objective: Genetic testing has become a routine part of the diagnostic workup in children with early onset epilepsies. In the present study, we sought to investigate a cohort of adult patients with epilepsy, to determinate the diagnostic yield and explore the gain of personalized treatment approaches in adult patients. Methods: Two hundred patients (age span = 18–80 years) referred for diagnostic gene panel testing at the Danish Epilepsy Center were included. The vast majority (91%) suffered from comorbid intellectual disability. The medical records of genetically diagnosed patients were mined for data on epilepsy syndrome, cognition, treatment changes, and seizure outcome following the genetic diagnosis. Results: We found a genetic diagnosis in 46 of 200 (23%) patients. SCN1A, KCNT1, and STXBP1 accounted for the greatest number of positive findings (48%). More rare genetic findings included SLC2A1, ATP6A1V, HNRNPU, MEF2C, and IRF2BPL. Gene-specific treatment changes were initiated in 11 of 46 (17%) patients (one with SLC2A1, 10 with SCN1A) following the genetic diagnosis. Ten patients improved, with seizure reduction and/or increased alertness and general well-being. Significance: With this study, we show that routine diagnostic testing is highly relevant in adults with epilepsy. The diagnostic yield is similar to previously reported pediatric cohorts, and the genetic findings can be useful for therapeutic decision-making, which may lead to better seizure control, ultimately improving quality of life.

Commentary

Identifying the cause of the most severe group of epilepsies, the developmental and epileptic encephalopathies (DEEs), seemed impossible only 20 years ago when these diseases were regarded as acquired. Over the last 2 decades, the number of genes that cause the DEEs has climbed exponentially, highlighting the not insignificant challenge of finding targeted therapies for each gene, let alone each specific molecular defect. There are now more than 400 genes associated with the DEEs. Exome

sequencing studies identify the causative pathogenic variant in around 50% of patients. With the molecular genetic revolution, the tables have turned, and the promise of finding the cause in all patients with DEEs is finally within sight.

DEEs are defined by a triad of features: (1) epileptic seizures (in most individuals), typically with multiple seizure types and uncontrolled by antiseizure medicines; (2) epileptiform activity on EEG, which is usually frequent; and (3) impact on development with slowing or regression.[1] The DEEs include a wide range of epilepsy syndromes, which may evolve from one to the other.[2] With increasing knowledge, each patient requires a diagnosis of both their genetic disease, such as *KCNQ2*-DEE, and epilepsy syndrome, such as Ohtahara syndrome. This is because both facets of the diagnosis have management implications. The genetic disease diagnosis informs selection of antiseizure medicines, recognition of comorbidities, and prognostic and genetic counselling. The epilepsy syndrome diagnosis also influences treatment choices and duration of treatment, assists with early recognition of seizure types and triggers, and may define prognosis. Neither of these diagnoses is adequate on their own because most genes have a spectrum of epilepsy presentations, so understanding each gene's phenotypic spectrum is key to providing tailored medical advice.

Increasing access to next generation sequencing in many regions of the world has changed the diagnostic landscape. Many families are now accessing exome sequencing, where the exons, the 1–2% of the genome that encodes proteins, are sequenced. A commonly used alternative is a gene panel where 10–600 genes relevant to the disorder of interest, such as epilepsy, are interrogated. Prior to sequencing, most patients should have a chromosomal microarray to exclude a pathogenic copy number variant.

The question is, for the remaining 50% of patients in whom a pathogenic variant has not been found, what is the next step in trying to find the cause of their severe disorder? Genome sequencing involves sequencing the whole genome, some 3 billion base pairs, to find pathogenic variants not present in the general population. This task is not for the faint-hearted as determining which one of the 4 million variants identified is causative for the disease phenotype is the challenge.[3]

Palmer and colleagues employed trio whole genome sequencing in 30 patients with DEEs.[4] Inclusion criteria required prior negative testing either by trio exome studies, where the exomes of the patient and parents were sequenced, or a multigene panel, following a normal chromosomal microarray and metabolic testing. Their molecular diagnosis rate was 63% with some important take-home messages. First and foremost, many pathogenic variants were identified by re-analysis and would equally have been ascertained by re-analysis of exome data. This occurred because of emerging evidence for pathogenicity for the gene or variant, due to publication of the gene, enlargement of its phenotypic spectrum, or new functional data. Their findings highlighted the importance of unbiased genomic data, showing a clear benefit of exome testing compared with the biased approach implicit in multigene panels that, by definition, cannot include all new emerging genes. Their yield is strikingly higher than the largest previous study of genome sequencing in 197 patients with DEEs that solved 32% only 3 years ago.[5] It is worth noting, however, that both studies only include unsolved cases, so do not provide an accurate epidemiological figure of the overall molecular yield for patients with DEEs.

Palmer et al applied a new *in silico* tool, ClinSV, to identify copy number and structural variants and detected 3/30 (10%) complex structural variants, missed by previous sequencing and clinical microarray. These complex structural variants were more challenging to detect because of their size, their balanced copy number (copy neutral), or their position effects. In two cases, the breakpoint of the structural variant lays within an established DEE gene. The third patient had a fascinating neutral copy number variant missed by exome sequencing as the proximal breakpoint was in a non-coding region. Further, it was hypothesized to disrupt a local topologically associated domain (TAD), which is a megabase region of DNA that comprises multiple loci that interact at high frequency and represents a fundamental functional unit of the genome.[6] TADs disrupting specific genes are associated with neurological diseases, emphasizing a novel 3D genomic structural mechanism likely to underlie some DEEs.

While the Palmer study finds an impressive diagnostic yield of >70% overall, how useful is genetic testing in the wider population with epilepsy? In particular, individuals with neurodevelopmental disorders have an increased risk of epilepsy. This group of patients, however, comprises 2 distinct groups—those with neurodevelopmental disorders and epilepsy without epileptic encephalopathy and those with DEEs. The former group is characterized by a static encephalopathy with developmental delay evolving to intellectual disability, where the epilepsy itself does not affect developmental progress or cognition.[7] A study of 200 Danish adults with epilepsy, mostly living in institutions, with >90% having intellectual disability, had a yield of only 23% by multigene panel testing.[8] In the 46 individuals with a molecular cause identified, the median age of seizure onset was 10 months, similar to pediatric studies; however, the range of seizure onset was 1 month to 23 years, highlighting the need to also study patients with later seizure onset.

Unsurprisingly, *SCN1A* was the most frequently identified gene (17 patients). Only 2/17 had a diagnosis of Dravet syndrome, highlighting the need to obtain infantile medical records in adults presenting with intellectual disability and epilepsy. Previous diagnoses in this cohort included focal epilepsy, Lennox–Gastaut syndrome, and unclassified DEE, whereas others have been erroneously diagnosed with vaccine encephalopathy.[9]

Johannesen and co-authors make the crucial point that a molecular diagnosis immediately allows clinicians to use a precision medicine approach, even in adults, improving quality of life and socioeconomic burden. For example, stopping the sodium channel blocker, carbamazepine, in a 41-year-old

woman with undiagnosed Dravet syndrome led to significant cognitive gains.[8] This emphasizes that clinicians are still missing the diagnosis of Dravet syndrome in infants and in older individuals with unacceptable consequences.[10]

Given that we can now identify the molecular basis in >70% of individuals with DEEs, solving 100% seems well within grasp, with life-changing implications for patients with the promise of precision therapies. The remaining 30% of patients is likely to have a pathogenic variant in yet-to-be-discovered genes, known DEE genes, non-coding regions, and through novel mechanisms involving transcriptional regulation and epigenetic factors.

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