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Clinical utility of genetic testing in pediatric drug-resistant epilepsy: A pilot study

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ABSTRACT

Rationale: The utility of genetic testing in pediatric drug-resistant epilepsy (PDRE), its yield in "real life" clinical practice, and the practical implications of such testing are yet to be determined.
Goal: To start to address the above gaps in our knowledge as they apply to a patient population seen in a tertiary care center.
Methods: We retrospectively reviewed our experience with the use of clinically available genetic tests in the diagnosis and management of PDRE in one clinic over one year. Genetic testing included, depending on clinical judgment, one or more of the following: karyotype, chromosomal microarray, single gene sequencing, gene sequencing panels, and/or whole exome sequencing (WES).
Results: We were more likely to perform genetic testing in patients with developmental delay, epileptic encephalopathy, and generalized epilepsy. In our unique population, the yield of specific genetic diagnosis was relatively high: karyotype 14.3%, microarray 16.7%, targeted single gene sequencing 15.4%, gene panels 46.2%, and WES 16.7%. Overall yield of diagnosis from at least one of the above tests was 34.5%. Disease-causing mutations that were not clinically suspected based on the patients' phenotypes and representing novel phenotypes were found in 6.9% (2/29), with an additional 17.2% (5/29) demonstrating pharmacologic variants. Three patients were incidentally found to be carriers of recessive neurologic diseases (10.3%). Variants of unknown significance (VUSs) were identified in 34.5% (10/29).
Conclusions: We conclude that genetic testing had at least some utility in our patient population of PDRE, that future similar larger studies in various populations are warranted, and that clinics offering such tests must be prepared to address the complicated questions raised by the results of such testing.

1. Introduction

Advancing technology in the areas of genetics and genomics has opened new avenues for diagnosis and understanding human disease. Tests that were previously solely the purview of research are now available commercially and, in some cases, direct-to-consumer [1]. Genetic testing can allow specific diagnoses and may influence therapy selection. For example, patients with sodium channel mutations such as in Dravet syndrome should generally avoid carbamazepine and lamotrigine [2], and genetic polymorphisms can predict the profile of drug metabolism and propensity for severe allergic reactions [3].

Identifying a specific genetic diagnosis can prevent further unnecessary and invasive diagnostic testing as well as allow genetic counseling for family members. But some clinicians argue that many of these clinical decisions can be arrived at through clinical data without expensive and confusing genetic investigations [4]. It has been recommended that genetic testing in epilepsy should be used in only select cases [5]. However, specific guidelines for when to apply genetic testing, the cost effectiveness of various testing modalities, and the clinical and practical implications of such studies are not available [6,7].

To start to address these questions, we reviewed in this study our experience with clinically available genetic studies and the implications of such testing on the diagnosis and management of pediatric drug-resistant epilepsy (PDRE) in our tertiary care center. Our goal was to describe, in retrospect, the yield of a variety of genetic testing modalities applied to our select referral population of patients.

2. Methods

In this retrospective chart review study, patients with PDRE were identified by reviewing records of all consecutive new patients seen in

Abbreviations: AR, autosomal recessive; PDRE, pediatric drug-resistant epilepsy; DRE, drug-resistant epilepsy; EE, epileptic encephalopathy; WES, whole exome sequencing; VUS, variant of unknown significance; AED, antiepileptic drug; IGE, idiopathic generalized epilepsy; LGS, Lenox–Gastaut syndrome; ESES, electrical status epilepticus of sleep; JME, juvenile myoclonic epilepsy.

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one pediatric epilepsy clinic during a 12-month period. Established patients who underwent whole exome sequencing (WES) during the study period were also included in the data collection (see Inclusion criteria below). The protocol was approved by the Duke IRB before starting the study.

2.1. Inclusion criteria

a. Patients with PDRE who were new to our clinic during the study period and who underwent genetic testing with one or more of the following tests (this included 25 out of 175 new patients seen in our clinic during that period of time, Fig. 1): karyotype, chromosomal microarray, gene sequencing of specific single genes, gene sequencing using gene sequencing panels, and/or WES. Microarrays were performed using the Affymetrix Cytoscan HD array, consisting of approximately 2.7 million markers incorporating 743,304 single nucleotide polymorphism probes and 1,953,246 nonpolymorphic copy number variation probes resulting in reporting of deletions >300 kb, duplications >500 kb, and regions of loss of heterozygosity of >10 Mb (Duke Clinical Laboratories). Gene panels included a commercially available infantile epilepsy panel which sequences 38 genes, childhood epilepsy panel which sequences 40 genes, and comprehensive epilepsy panel which sequences 53 genes, all performed by GeneDx (Gaithersburg, MD). Epilepsy associated with fever in females' panel evaluation was also used and covers 4 genes (Athena Diagnostics, Worcester, MA). Mitochondrial genome sequencing was ordered for one patient (Baylor College of Medicine Medical Genetics Laboratories, Houston, TX). Whole exome sequencing was performed through Baylor Laboratories (Houston, TX). Further details regarding the technical performance of these commercial CLIA lab-based tests can be found on the respective lab websites. All patients were counseled about the testing before it was performed and were referred after the results became available for further genetic counseling in the genetics clinic. The order in which tests were sent depended on the clinical scenario and clinical judgment at the time. For example, if a patient was suspected to have Dravet syndrome, SCN1A or febrile epilepsy gene panel sequencing was ordered without obtaining microarray or karyotype. For patients with generalized epileptic encephalopathy, microarray and gene panels were often ordered together.
b. Patients with DRE who initially had been seen in our clinic prior to the availability of WES and who had WES performed during the study period. Inclusion of these patients allowed us to better investigate the potential utility and yield of WES in PDRE. This group included four patients who were combined with the two new patients who underwent WES as well. However, these four established patients were not included in the analysis of factors associated with yield of genetic testing so as not to bias the analysis which included only newly encountered consecutive patients during the one-year study period.

2.2. Data collected

Collected clinical data included the following:

a. Patient age at initial evaluation in our clinic, age at first seizure reported at the initial encounter, gender, seizure types, epilepsy syndromes, comorbidities, level of developmental delay/intellectual disability when present, presence or absence of history of developmental regression, medications taken at the time of initial evaluation, and previously taken antiepileptic drugs (AEDs) discontinued prior to our first evaluation. We also collected data on EEG and neuroimaging findings.
b. Findings of genetic testing based on classification listed below.

2.3. Definitions and classifications

For the purposes of our study, we used the following definitions and classifications:

a. Drug-resistant epilepsy was defined according to the International League Against Epilepsy (ILAE) as failure of two appropriate AEDs to control seizures [8]. Discontinuation of a drug due to side effects was not considered a failure. Appropriateness of intervention was based on historical and clinical data available at the time of the visit to our clinic.
b. Severity of developmental delay or intellectual disability was rated by the following scale adapted from a previous study [9]: mild for delays/disabilities that may only be apparent after starting school with communication skills that could lead to employment and independent living, moderate for patients with some difficulty with motor skills and speech but able to communicate through verbal means, and severe for patients with marked limitations in motor skills and language ability.
c. Epileptic encephalopathy was diagnosed, according to the ILAE definition, when epileptiform EEG abnormalities were thought to contribute to a patient's cognitive impairment [10].
d. For the purposes of our study, we classified results of genetic testing according to the following: 1) normal (negative result), when there were no mutations or polymorphisms or when there were only polymorphisms known to be normal variants, and 2) positive, when there was a reportable finding with potential clinical significance. Here we distinguished between a positive result (potentially significant) and diagnostic result (indicative of a specific diagnosis), the latter being a subcategory of the former. Positive results consisted of the following four categories of reportable findings reported by the CLIA-certified laboratories. a.) Diagnostic results, which consisted of mutations previously described to be disease-causing for epilepsy syndromes or variants predicted to be disease-causing. Predictions were made by the CLIA-certified laboratories and were included in the results reported to the ordering physician [11]. Predictions are based on protein structure and function and level of sequence conservation across species. Scores based on in silico models were usually not reported by the lab, and only a designation as “disease-causing” or, in one patient (see the Results section and Table 4), “likely disease-causing” was reported in these cases. For autosomal recessive disorders, two disease-causing mutations had to be present for the mutation to be considered diagnostic, none of which were found in our patients. b.) Heterozygosity for an autosomal recessive neurologic condition. Although heterozygous mutations for autosomal recessive (AR) diseases are unlikely to be disease-causing unless there is an undetected mutation in the other allele, these results are reported as positive as they have genetic counseling implications and may, in rare cases, contribute to the disease process. c.) A variant known to affect pharmacokinetics or pharmacodynamics, which is a finding that can contribute to clinical management when the altered gene is involved in AED pharmacology. d.) Variants of unknown significance in genes related to neurologic disease. While VUS could not be assumed to contribute to the patient's disease, they did require interpretation for the family and may, in some cases, be found to be clinically significant in the future as new data become available. Since this is a retrospective clinical study, we have described the results as they were reported by the CLIA-certified laboratory.
e. We considered a mutation to be unexpected if 1) an epilepsy-related gene carried a mutation while the patient's phenotype differed from the phenotype considered typical of the identified mutation and thus would not have led to targeting that specific gene for sequencing had a panel or WES not been performed and 2) a variant known to affect drug pharmacokinetics or pharmacodynamics was identified that could not have been identified had a WES not been requested.