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Genetic Diagnosis Impacts Medical Management for Pediatric Epilepsies

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Abstract

Background: Evidence of the impact of genetic diagnosis on medical management in individuals with previously unexplained epilepsy is lacking in the literature. Our goal was to determine the impact of genetic diagnosis on medical management in a cohort of individuals with early-onset epilepsy.

Methods: We performed detailed phenotyping of individuals with epilepsy who underwent clinical genetic testing with an epilepsy panel and/or exome sequencing at Boston Children's

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Supplementary data

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Hospital between 2012 and 2019. We assessed the impact of genetic diagnosis on medical management.

Results: We identified a genetic etiology in 152 of 602 (25%) individuals with infantile- or childhood-onset epilepsy who underwent next-generation sequencing. Diagnosis impacted medical management in at least one category for 72% of patients (110 of 152) and in more than one category in 34%. Treatment was impacted in 45% of individuals, including 36% with impact on antiseizure medication choice, 7% on use of disease-specific vitamin or metabolic treatments, 3% on pathway-driven off-label use of medications, and 10% on discussion of gene-specific clinical trials. Care coordination was impacted in 48% of individuals. Counseling on a change in prognosis was reported in 28% of individuals, and 1% of individuals had a correction of diagnosis. Impact was documented in 13 of 13 individuals with neurotypical development and in 55% of those with epilepsy onset after age two years.

Conclusion: We demonstrated meaningful impact of genetic diagnosis on medical care and prognosis in over 70% of individuals, including those with neurotypical development and age of epilepsy onset after age two years.

Keywords

Epilepsy; Next-generation sequencing; Whole exome sequencing; Precision treatment; Development

Introduction

Early-onset epilepsies are common neurological disorders that occur in approximately seven of 10,000 infants per year,[1,2] and genetic factors are thought to play a major role in cases without a clear structural or metabolic etiology.[1,3–6] Single-gene etiologies can be diagnosed in approximately 25% to 30% of individuals with unexplained epilepsy, and in up to 55% to 80% of those with neonatal-onset epilepsy and developmental and epileptic encephalopathies (DEEs).[3–9] In the more common epilepsies, heritability data suggest polygenic contribution to risk, but there is also evidence of higher burden of rare variants in known epilepsy genes.[10,11]

Epilepsy gene panels and exome sequencing (ES) have been increasingly utilized for precision clinical diagnosis in epilepsy. Beyond the benefits of diagnosis and related genetic counseling, specific treatment approaches are indicated for an increasing number of genetic epilepsies.[12–18] Genetic diagnosis may also lead to more specific information regarding prognosis, such as rates of sudden unexpected death in epilepsy or a progressive disease course, or the need to screen for associated conditions (e.g., renal or cardiac disease).[13,14,19,20] However, evidence of the impact of genetic diagnosis on management of epilepsy in a clinical setting to establish the utility of genetic testing in epilepsy is a critical area of unmet need, highlighted in a systematic review of genetic testing for the epilepsies.[9,15,19–21]

In this study, we sought to assess the impact of genetic diagnosis on medical management, as well as to determine phenotypic predictors associated with diagnosis and medical management impact.

Materials and Methods

Cohort and genetic testing

This is a retrospective cohort study approved by the Boston Children's Hospital (BCH) Institutional Review Board. We evaluated a cohort of individuals seen at BCH who had a clinical epilepsy gene panel result from GeneDx, the major commercial laboratory with whom BCH contracted during this time frame, between June 2012 and January 2019. These individuals were identified from a list provided by GeneDx. Inclusion criteria included epilepsy gene panel report and diagnosis of epilepsy[22,23] confirmed by retrospective medical record review. A subset of individuals underwent either clinical or research ES. Clinical ES was based on clinician referral, typically following nondiagnostic initial genetic testing. Research exomes through the Children's Rare Disease Initiative or the BCH Epilepsy Genetics Program were offered for individuals with unexplained epilepsy, including those with a negative clinical genetic evaluation, those for whom insurance did not cover clinical testing, and epilepsies for which clinical genetic testing was not standard of care (e.g., benign epilepsy syndromes).[24]

Genetic testing classification

Epilepsy panel or ES results were considered diagnostic if variants identified on initial testing or reanalysis were interpreted by the treating team to explain the patient's epilepsy.[24] The majority of diagnostic variants were pathogenic or likely pathogenic based on classification per American College of Medical Genetics (ACMG) Guidelines.[25] The exceptions to this rule were as follows: (1) the laboratory classification was variant(s) of unknown significance (VUS) or variant(s) in candidate genes, but literature after the laboratory report led to reclassification as disease-associated and upgrade of the variant(s) to (likely) pathogenic; and (2) the variant remained a VUS but the result was considered diagnostic by expert clinical diagnosis with unique clinical findings not captured by ACMG criteria (Table 1). Epilepsy panels and ES were considered nondiagnostic if they were negative, included VUS other than the aforementioned ones, or identified variants in gene(s) not consistent with the patient's epilepsy phenotype or the condition's mode of inheritance.

We additionally collected data on chromosomal microarray results and other diagnoses (e.g., hypoxic-ischemic encephalopathy) contributing to epilepsy, both genetic and nongenetic.

Clinical phenotyping

To determine potential clinical predictors of diagnostic yield, we identified clinical features prevalent in DEEs from medical records. We collected seizure data, including epilepsy type, seizure patterns and types, electroclinical syndrome, and electroencephalography (EEG) encephalopathy pattern, according to International League Against Epilepsy standards.[22,23] Epileptic encephalopathy patterns as mentioned in EEG clinical reports were categorized as burst suppression, hypsarrhythmia (full or modified), generalized slowing with multifocal and/or generalized epileptiform activity, slow spike and wave ± fast activity, electrical status epilepticus in sleep, and other (Table S1). We additionally collected demographic, developmental, and examination features (Tables 2 and 3, Table S1).

The concept of DEE recognizes that "in infants presenting with severe early-onset epilepsy, neurodevelopmental comorbidity may be attributable to both the underlying cause and to the adverse effects of uncontrolled epileptic activity."[22,26,27] In this cohort of individuals with early-onset epilepsy with suspected or confirmed genetic etiology and related developmental impairments in whom other etiologies for epilepsy were excluded, we designated those with a combination of epilepsy onset <18 years, encephalopathy pattern on EEG, and developmental delay (DD) or intellectual disability (ID) as having DEE. We defined status epilepticus as continuous seizure or multiple seizures without recovery, lasting for 30 minutes or longer.[28] Cortical visual impairment was defined as "visual dysfunction in the absence of ocular or anterior visual pathway abnormalities."[29]

Medical management

The electronic medical records of individuals with positive genetic test results (n = 152) were reviewed by an MD independent of the BCH clinical Epilepsy Genetics Program (I.H.) to establish individualized impact on medical management and verified by a pediatric neurologist with expertise in epilepsy genetics (H.E.O.). We identified four categories of medical management: treatment impact, which includes choice of antiseizure medications (ASMs), gene-specific vitamin/metabolic treatments, pathway-driven off-label use of medications, and disease/gene-specific clinical trials; care coordination, which includes medical management and monitoring for disease-associated features, including specialist referrals (except genetics), surveillance through diagnostic testing, and referrals to disease-specific clinics; change in prognosis; and correction of diagnosis (Table 4).

Statistical analysis

We first evaluated the overall diagnostic yield of clinical testing for epilepsy by gene panel with or without ES.

Second, we evaluated diagnostic yield for phenotypic subsets based on clinical features, including those with autism spectrum disorder (ASD), ID, global developmental delay (GDD), neurotypical development (defined as no diagnosis of DD, ASD, or ID), developmental regression, head size, systemic malformations, movement disorders, and those with DEE. Pearson chi-square test or Fisher exact test was performed to examine the distribution of phenotypic features on diagnostic yield.

Third, we used multivariate logistic regression modeling to assess (1) clinical factors predictive of genetic diagnosis by gene panel, ES, or a combination; (2) clinical factors predictive of diagnostic ES after negative epilepsy gene panel; and (3) clinical factors predictive of impact of diagnosis on medical management. Statistical analysis was done using SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA). The outcome variable for the first analysis was diagnostic panel and/or ES, and in the second analysis, diagnostic ES. Early age of epilepsy onset (particularly in the neonatal and infantile period up to two years) and developmental disorder are previously described predictors of genetic diagnosis[5,16,30–32] and were considered in the multivariate model as possible predictors of impact of genetic diagnosis on medical management. Based on this prior literature and consistent with the International League Against Epilepsy age cutoff of two years for the neonatal and infantile

period,[26] we used epilepsy onset less than or equal to two years in the model. Predictor variables were analyzed using univariate testing of Wald tests, as well as box-and-whisker plot and Wilcoxon rank-sum test for epilepsy age of onset. Each predictor was examined alone in a univariate logistic model. A multivariate logistic regression model was then built, keeping age of epilepsy onset as a key predictor in each model based on preliminary data and prior literature.[5,16,30–33] A threshold *P* value of 0.25 was used to select candidate predictor variables, and we then used a backward selection approach including variables with a *P* value of < 0.05 in the final model.

Results

Our cohort consists of 602 individuals (278 female) with early-onset epilepsy (seizure onset median 1.0 years; interquartile range [IQR] 4 months, 3.0 years), who had a clinical epilepsy gene panel result at BCH between June 2012 and January 2019. The most commonly used gene panels included the comprehensive epilepsy panel (n = 411) and infantile epilepsy panel (n = 129) (Table S1). A subset with nondiagnostic panel results subsequently underwent clinical or research ES (n = 183) (Fig 1). Our cohort includes 318 of 600 individuals (53.0%, 2 unknown) meeting criteria for DEE,[22,26,27] defined as age of epilepsy onset <18 years, encephalopathy pattern on EEG, and DD and/or ID. Epilepsy was generalized in 32.8%, focal in 32.6%, mixed in 28.0%, and unknown in 6.7%. Of individuals with diagnosed electroclinical syndromes (n = 190), the most common included West syndrome (n = 36, 18.9%) and Lennox-Gastaut syndrome (n = 29, 15.3%). Median age at last follow-up was 8.2 years (IQR 4.2 years, 12.9 years). Our cohort is 70.6% white and 75.9% non-Hispanic (Table 2).

The overall diagnostic yield of clinical testing for epilepsy by gene panel with or without ES was 25.3% (152 of 602 individuals) (Fig 1). This included 15.8% (95 of 602) yield for epilepsy gene panel, with highest yield for the STAT panel (five of 14) and infantile panel (39 of 129) and no definite increase from 2012 to 2019; 36.7% (40 of 109) yield for clinical ES initial analysis after nondiagnostic panel; 21.4% (three of 14) yield for clinical ES reanalysis after non-diagnostic clinical ES; and 10.9% (11 of 101) diagnosed by clinical confirmation of research ES result after initial negative clinical testing (Fig 1). Causative variants were pathogenic or likely pathogenic according to ACMG classification except for those of eight individuals for whom genetic variants remained VUS after ACMG reclassification (Table 1). Eight individuals (1.3%) had a partial or whole gene deletion or duplication that would not have been identified by ES in the time frame of the study.

Of the 152 individuals with a genetic diagnosis, genes in which causative variants were most commonly identified on both panels and exomes included *SCN1A* (n = 19), *KCNQ2* (n = 12), *PRRT2* (n = 8), *SCN2A* (n = 7), *CDKL5* (n = 6), *CHD2* (n = 5), *TSC2* (n = 4),*STXBP1* (n = 4), and *SCN8A* (n = 4) (Fig 2). Of the 54 individuals with a diagnostic exome (clinical or research-based) following a non-diagnostic gene panel, causative variants were reported in the following genes in more than one individual: *DYNC1H1* (n = 3), *ALG11* (n = 2), *CHD2* (n = 2), *FRRS1L* (n = 2), *NEXMIF* (n = 2), *PACS2*(n = 2), *SCN8A* (n = 2), and *WDR45* (n = 2) (Fig 3). Additional genetic details are provided in Table S3.

Although this is largely a nonmalformation-related epilepsy cohort, in the case of variants in genes known to be associated with brain malformations, including *DYNC1H1, FOXG1*, *KANSL1*, *TSC2*, *TUBB2A*, and *ZEB2*, the patterns of malformation identified were consistent with expectations for the genetic diagnoses. In two individuals with *DYNC1H1* variants, a malformation was identified only on detailed review after genetic diagnosis (one after statistical analysis, recognized only following research review for this study). There were also malformations identified in individuals with nonmalformation-related genetic diagnoses, including *SCN1A* and *AGO1* diagnoses with cortical dysplasia, *SCN8A* diagnosis with mild inferior vermian hypoplasia, *GRIN2A* diagnosis with prominent thalamic adhesion, and *CDKL5* diagnosis with a cortical heterotopia.

Yield of diagnosis by epilepsy gene panel and/or ES by predefined phenotypic features included the following: ASD (if age greater than or equal to three years) 22.9%, ID (if age greater than or equal to five years) 25.9%, GDD (if age greater than or equal to two years) 31.7%, neurotypical development (if age greater than or equal to two years) 8.8%, and DEE 30% (Tables 3 and S2). Comparing the cohort of individuals with ES after a nondiagnostic panel (n = 183) with those without ES after a negative panel (n = 310), age of epilepsy onset was less than or equal to two years in 69.4% and 57.1%, respectively; ASD in 31.4% and 18.6%, respectively; GDD in 69.4% and 50.9%, respectively; abnormal muscle tone (hypotonia, spasticity, dystonia, or mixed patterns) in 58.5% and 37.7%, respectively; and cortical visual impairment in 23.2% and 11.9%, respectively.

Using multivariate regression analysis, positive or negative predictors of genetic diagnosis by epilepsy gene panel, ES, or a combination included age of epilepsy onset less than or equal to two years (odds ratio [OR], 3.82; 95% confidence interval [CI], 2.19,6.66), malformation of brain development (OR, 0.27; CI 0.15, 0.51), focal motor seizures (OR, 2.29; CI, 1.43, 3.65), and DD (OR, 2.64; CI, 1.47, 4.73) (Tables 5 and S2). Controlling for malformation of brain development, seizure types, and DD, the odds of a diagnostic test result were 3.82 times higher (CI, 2.19, 6.66) in individuals with epilepsy onset less than or equal to two years compared with individuals with later epilepsy onset.

All individuals with diagnostic ES after a negative epilepsy panel had DD in at least one area, so GDD was used for statistical modeling. Using multivariate regression analysis, features predictive of genetic diagnosis by ES after a negative epilepsy panel were abnormal muscle tone (OR, 3.02; CI, 1.20, 7.59) and GDD (OR, 4.45; CI, 1.14, 17.31) (Table 5 and S2). Age of epilepsy onset was required in the model but was below significance in the final model ($P = 0.12$; OR, 2.23; CI, 0.81, 6.19). Controlling for age of epilepsy onset less than or equal to two years and GDD, the odds of diagnostic ES after a negative panel were 3.02 times higher (CI 1.20, 7.59) in individuals with abnormal muscle tone compared with individuals with normal muscle tone. There is evidence of colinearity of age of epilepsy onset less than or equal to two years with GDD and abnormal muscle tone. If GDD is removed from the model, age of epilepsy onset less than or equal to two years is a significant predictor of diagnostic ES (OR, 3.21; CI, 1.22, 8.44), $P. = 0.02$, and the P value for tone decreases to 0.0003 (Table 5).

Genetic diagnosis had a direct impact on medical management for 72.4% of individuals with a diagnostic result (110 of 152), including 33.6% (51 of 152) with impact in more than one category. No impact on medical management was documented for 22.3% of individuals (34 of 152), including for four individuals who were deceased before the availability of genetic results. Median length of follow-up after genetic diagnosis was 1.7 years (IQR 0.3, 3.8). Impact on medical management was unknown in eight individuals who were not followed after diagnosis (5.2%). According to specific medical management categories, 45.4% of individuals were found to have impact on treatment (69 of 152), including 35.5% on choice of ASMs, 6.6% on use of disease-specific vitamin or metabolic treatments (including ketogenic diet), 3.3% on pathway-driven off-label use of medications, and 9.9% on discussion of disease or gene-specific clinical trials between family and clinicians or investigational new drug use. Examples are provided in Table 4 and detailed for all participants in Table S4.

We documented impact on care coordination in 48.0% of individuals (73 of 152), including surveillance for other disease-associated features (including non-neurological features), additional disease-specific diagnostic testing, specialist referrals, and referrals to disease-specific clinics (e.g., tuberous sclerosis complex, CDKL5 deficiency disorder) (Tables 4 and S5). Although we did not systematically collect information on outcomes of testing and specialist evaluation as some individuals were not followed at BCH, there were instances in which referrals led to identification of disease-associated features (e.g., *TRIM8* variant led to nephrology referral and diagnosis of nephrotic syndrome and focal segmental glomerulosclerosis). In 27.6% of individuals, we documented counseling related to change in prognosis or life expectancy, such as discussion of degenerative diseases (e.g., *NHLRC1*-related Lafora disease), risk for early mortality or sudden unexpected death in epilepsy (e.g., *BRAT1, SCN1A*), and optimistic long-term outcomes (e.g., *PRRT2*). Although most participants lacked a diagnosis before genetic testing, 1.3% of individuals had a correction of diagnosis, replacing a nonspecific diagnosis of mitochondrial disorder with a precise genetic diagnosis (*GNAO1, CACNA1A*). For 73.0% of individuals (n = 111), there exists a gene-specific family organization related to their diagnosis. Of those with a genetic diagnosis and data available on medical management impact (n = 144), impact was documented in 55% of those with epilepsy onset greater than two years (12 of 22), compared with 80% of those with onset at less than or equal to two (98 of 122) (chi-square test, $P = 0.0088$). Medical management was impacted in 73.5% of those with a developmental disorder (DD, ASD, and/or ID) (97 of 131) and all 13 individuals with neurotypical development (Fisher exact test, $P = 0.0386$).

Discussion

Overall, as genetic testing practices evolved from 2012 to 2019, we identified genetic diagnoses in 152 (25.3%) of 602 individuals with early-onset epilepsy of whom over 70% had direct impact on medical management. Expanding on prior reports establishing yield of genetic diagnosis in epilepsy with limited information on direct medical impact, this study emphasizes direct clinical utility.[9] Furthermore, although medical impact was higher in those with epilepsy onset under two years, impact was found in over 50% of individuals with epilepsy onset after age two years and in all 13 individuals with neurotypical development.

We observed age of onset less than two years, DD, and focal motor seizures to be phenotypic features associated with higher odds of genetic diagnosis. Furthermore, we demonstrate meaningful additional yield of clinical ES after a negative epilepsy gene panel (39.5%), especially in the setting of GDD and abnormal muscle tone. The yield of diagnosis by panel or ES in our study was similar to the yield reported in prior literature that included broad cohorts of individuals with epilepsy.[9,34] Genes for which causative variants were most commonly identified in our cohort closely matched the genes most commonly implicated in epilepsy in other series of next-generation sequencing.[34,35] Genes for which variants were identified on ES after a negative panel in this cohort similarly included genes coding for proteins and pathways known to be linked to epilepsy and brain malformations and consistent with prior literature, although in many cases not typically included in epilepsy panels (e.g., congenital disorders of glycosylation).[30,34]

In recent years, clinical genetic testing in neurology has been shifting from panel testing to an exome-first approach at some larger academic centers with adequate genetic counseling resources, as supported by literature for neurodevelopmental disorders and by a cost-effectiveness study in epilepsy genetics.[36,37] Findings on panel testing that would not have been expected to be identified by ES were seen in the eight of 602 individuals in our cohort (1.3%) with copy number variants identified by epilepsy panel. Between 2012 and 2019, Copy nuber variant analysis was not routinely performed on exomes, but it is now an emerging practice. Thus, given the significant additional yield of ES over epilepsy gene panel, improvement of certainty in variant classification with a trio ES approach, the ability to reanalyze data over time, and the falling costs of ES, we suggest ES as an appropriate first-line test in the diagnosis of unexplained epilepsy. If ES is performed and not revealing of a definite etiology, we suggest consideration of exon-level copy number variant assessment.

This is the first study to demonstrate the direct medical impact of genetic diagnosis in epilepsy in a clinical cohort, providing evidence of consequent tailored care in more than 70% of individuals, including impact on treatment, care coordination, prognosis, and correction of diagnosis. Although prior studies have reported the impact of ES on medical management in broader pediatric populations with a variety of diagnoses, as well as theoretical impact of genetic diagnoses on medical management in epilepsy,[19–21,34,36,38–42] these reports did not evaluate individualized medical management impact as we have done in this current large clinical series. Over one-third of participants had documented impact on ASM choice, and we demonstrate increasing impact of pathway-driven treatments and experimental therapies with improved precision diagnosis. Results are consistent with impacts of the highest-yield genes on recommended treatment approaches[38–42] as well as novel approaches to precision treatment and identification of new genes in treatable pathways.[12–18,43] Reported percentages may be an underestimate, because some individuals were seen only for a consult at BCH but were followed elsewhere. Furthermore, lack of documented medical impact in 30 individuals (excluding four participants who passed away before genetic results) does not preclude the possibility of future impact for these recently reported genetic diagnoses when more information becomes available.

In addition to the documented impact on medical management, families routinely received genetic counseling consultations, which include discussion of recurrence risk, reproductive planning, cascade testing of at-risk family members, and referral to gene-specific family advocacy organizations. For those with a genetic diagnosis without an existing family advocacy organization, our Epilepsy Genetics Program informs families about the Rare Epilepsy Network (REN), Syndromes Without A Name (SWAN), Genome-Connect, and applicable social media groups and utilizes GeneMatcher.

Although prior reports have shown a correlation between age of epilepsy onset, presence of comorbid neurodevelopmental features, and diagnostic yield,[5,16,30–32] these features have not been previously studied in relation to medical impact. Our results show that although early age of epilepsy onset (less than or equal to two years) may be associated with an increased likelihood of medical impact, impact was nevertheless noted in >50% of those with onset greater than two years. Impact was significant both in those with developmental disorders and neurotypical development.

There are limitations inherent to our retrospective study design, including inability to systematically collect outcomes of surveillance testing and referrals. EEG patterns and developmental diagnoses were reported as documented in medical records. Because DEE was only defined during the course of this study and not consistently documented in medical records, we defined a triad of features that are suggestive of this diagnosis in this particular population with epilepsy of suspected genetic etiology. Our sample size for evaluating predictors of genetic diagnosis by ES after a negative panel was low, and additional predictors may have reached significance with higher power. Phenotypic features such as ID and ASD were not identified as significant predictors of genetic diagnosis, possibly related to limited sample size; requirement for age at last follow-up after five years and three years, respectively; and confounding with other significant predictors. Age of epilepsy onset under two years was not a significant predictor of diagnostic ES after negative gene panel likely due to confounding with abnormal tone and GDD. We suspect that focal motor seizures were a significant predictor of diagnosis because it is a common seizure type in neonatal-onset epilepsy. There was clinical selection bias in those who went on to ES for individuals with a broader neurodevelopmental disorder, and the yield of ES for the entire cohort may have been lower. Yield of research exomes was lower than for clinical exomes, likely explained by ongoing data analysis and selection bias due to inclusion of individuals with benign epilepsy syndromes and others with prior negative clinical genetic testing.

This study was conducted during a time period characterized by a shifting landscape of genetic testing in epilepsy. Although this study included multiple types of epilepsy panels with gene lists that varied in size and over time, the yield did not substantially increase over the time of the study and the highest yield was seen in the panel types sent in individuals with infantile-onset epilepsy. Testing in the earlier years, however, was likely influenced by selection bias, with more severely affected individuals with epilepsy sequenced, compared with more recent years, when genetic testing in epilepsy has been applied to a broader group of patients with epilepsy at BCH. We used data primarily from one diagnostic laboratory due to institutional contracting with our hospital. We do not have reason to believe results would be significantly different with different diagnostic laboratories, but it may have been

higher with use of larger panels. Last, the majority of individuals in our cohort were white and non-Hispanic. Similar evaluations in other race and ethnic groups would be beneficial.

By including individuals with an epilepsy gene panel rather than ES only or brain malformation panels, our cohort focused on those with nonmalformation-related epilepsy as the predominant phenotype. Brain malformations were a negative predictor of genetic diagnosis in our series, which can be explained by the fact that epilepsy panels are targeted to nonmalformation-related epilepsy. Malformations identified were consistent with genetic diagnosis or, rarely, found nonspecifically in association with nonmalformation-related epilepsy genes such as *SCN1A* as has been reported.[38,44,45]

In conclusion, our study demonstrates substantial impact of genetic diagnosis on medical management in individuals with epilepsy. The likelihood of a diagnostic genetic test was highest in individuals with DD, abnormal muscle tone, and focal motor seizures. Medical impact is relevant for both individuals with neuro-typical development and with developmental disorders, and regardless of age of seizure onset. This study supports the inclusion of genetic testing, ES in most clinical scenarios, as part of the standard evaluation for individuals with unexplained epilepsy as a means of achieving diagnostic precision and potentially informing clinical management.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Potential conflicts of interest:

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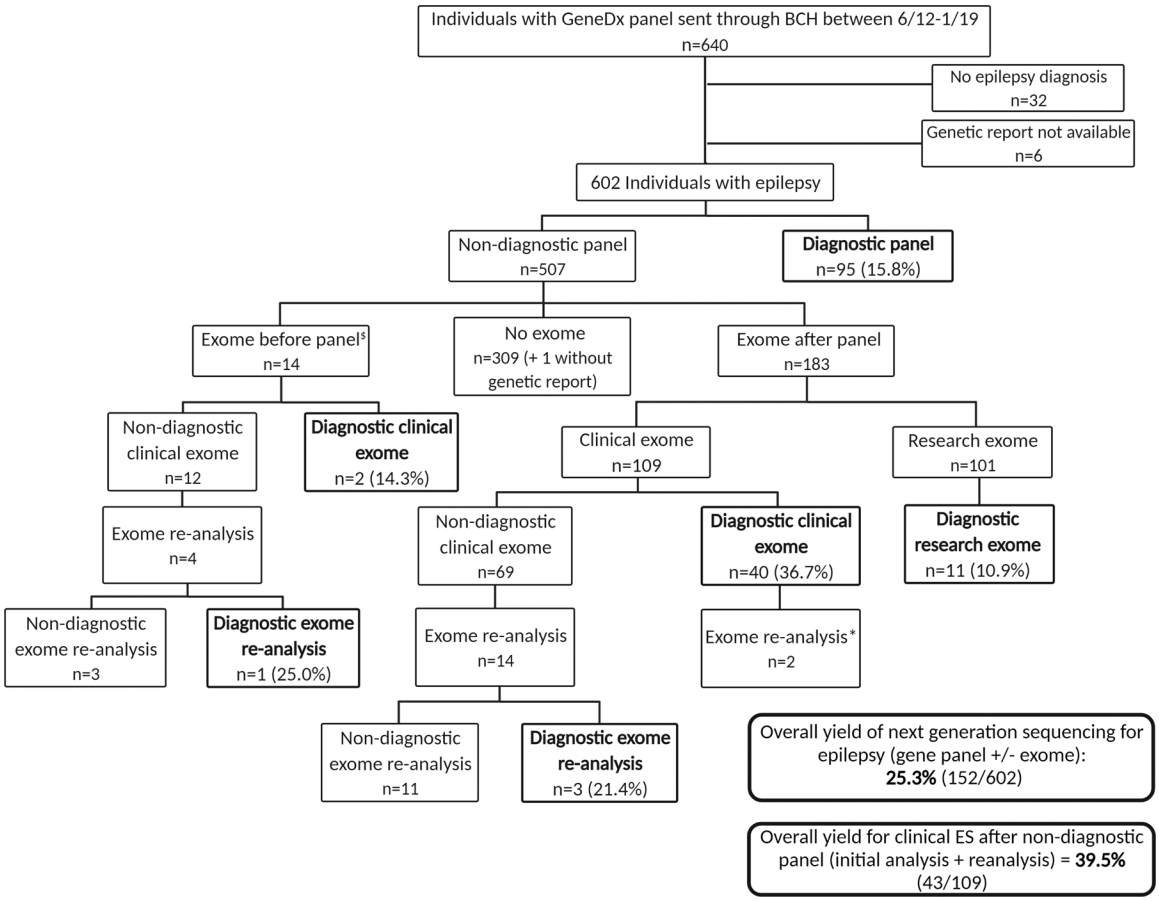
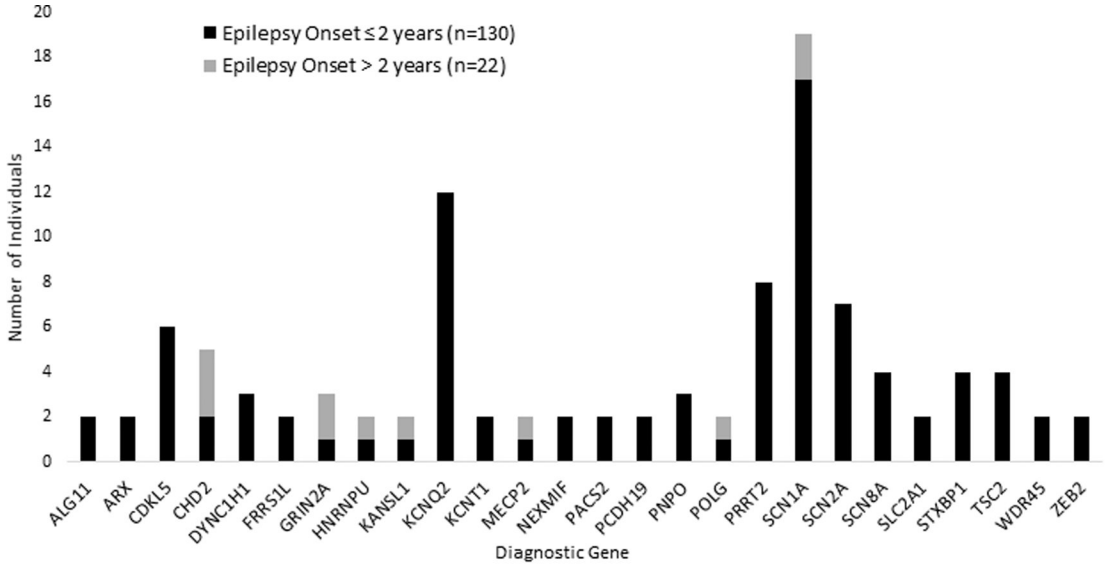


FIGURE 1.

Study flowchart. Inclusion/exclusion criteria, genetic testing algorithm, and results of our cohort. Figure created with BioRender.com. *For two individuals, exome reanalysis was done after clinical exomes with diagnostic findings in AGO1 and CHAT to look for further contribution to epilepsy phenotypes that were more severe than expected. No additional findings were identified. $In 13 of 14, panel was done to expand genetic evaluation when exome was nondiagnostic or did not fully explain the phenotype, including full coverage of updated epilepsy genes and copy number variant analysis. In one additional patient, rapid exome was sent while panel was pending due to severity of the medical condition.

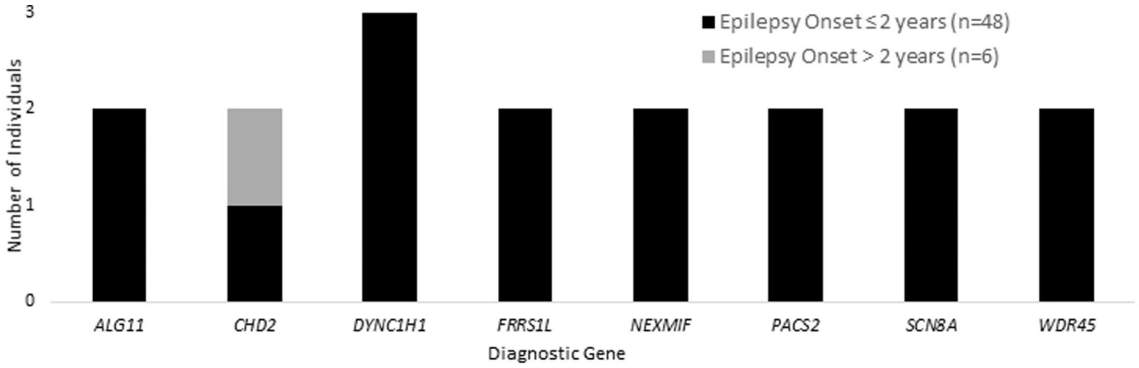


Additional genes for which variant(s) associated with epilepsy were identified, epilepsy onset ≤ 2 years (n=1 each)									
ACTB	*AGO1*	*AP4S1*	*ARHGEF9*	*ATP1A3*	*ATP6V1A*	*BRAT1*	*CACNA1A*	*CACNA1E*	*CHAT*
CNTNAP2	*COL4A1*	*DLL1*	*ERCC5*	*FOXG1*	*GABRB2*	*GABRG2*	*GNAO1*	*IFIH1*	*IQSEC2*
ITPA	*KCNH1*	*NRXN1/2*	*PLPBP*	*RHOBTB2*	*SCN1B*	*SEPSECS*	*SLC12A5*	*SPTAN1*	*TBL1XR1*
TRIM8	*TUBB2A*	*UBE3A*	*UPF3B*	*WDR73*					

Additional genes for which variant(s) associated with epilepsy were identified, epilepsy onset > 2 years (n=1 each)									
CHRNA7	*EEF1A2*	*KCNMA1*	*MEF2C*	*NBEA*	*NHLRC1*	*NPRL2*	*NRXN1*	*SHANK3*	*SYNGAP1*
TPP1									

FIGURE 2.

Implicated genes for epilepsy diagnosis in a clinical cohort of 602 individuals with infantile- or childhood-onset epilepsy from Boston Children's Hospital. Genes with variants identified on panel or exome thought to clinically explain epilepsy for 152 individuals, 130 with epilepsy onset less than or equal to two years of age. The most common genes for which causative variants were identified included *SCN1A* (n = 19), *KCNQ2* (n = 12), *PRRT2* (n = 8), *SCN2A* (n = 7), *CDKL5* (n = 6), *CHD2* (n = 5), *TSC2* (n = 4) *STXBP1* (n = 4), *SCN8A* (n = 4) *CHD2* and *SCN8A*, which were later added to panels, were identi fied on both panels and exomes in our series.



Additional genes for which variant(s) associated with epilepsy were identified, epilepsy onset ≤2 years (n=1 each)									
ACTB	*AGO1*	*AP4S1*	*ARHGEF9*	*ATP1A3*	*ATP6V1A*	*BRAT1*	*CACNA1E*	*CDKL5*	*CHAT*
COL4A1	*DLL1*	*GABRB2*	*GNAO1*	*HNRNPU*	*IFIH1*	*IQSEC2*	*ITPA*	*KCNH1*	*KCNT1*
NRXN1/2	*PLPBP*	*RHOBTB2*	*SEPSECS*	*SLC12A5*	*SPTAN1*	*TBL1XR1*	*TRIM8*	*TSC2*	*TUBB2A*
UPF3B	*WDR73*								

Additional genes for which variant(s) associated with epilepsy were identified, epilepsy onset >2 years (n=1 each)									
KCNMA1	*NBEA*	*NPRL2*	*SHANK3*	*SYNGAP1*					

FIGURE 3.

Implicated genes for epilepsy diagnosis in a clinical cohort of 183 individuals with infantile- or childhood-onset epilepsy from Boston Children's Hospital and initial negative epilepsy gene panel testing. Genes with causative variants identified on clinical or research exome, following a nondiagnostic panel, in 54 individuals with epilepsy, 48 with age of epilepsy onset less than or equal to two years. The genes for which causative variants were identified in more than one individual included DYNC1H1 (n = 3), *ALG11*(n = 2), *CHD2* (n = 2), *FRRS1L* (n = 2), *NEXMIF* (n = 2), *PACS2* (n = 2), *SCN8A* (n = 2), and *WDR45* (n = 2).

Table 1.

Race and ethnicity data for 602 individuals with an epilepsy panel with or without exome sequencing, sent through BCH between June 2012 and January 2019.

Racial Category	**Hispanic or Latino**	**Not Hispanic or Latino**	**Unknown/Not Reported**	**Total n (%)**
American Indian or Alaskan Native	1	2	0	3 (0.5)
Asian	1	40	1	42 (7.0)
Black or African American	2	30	8	40 (6.6)
Native Hawaiian or Other Pacific Islander	1	3	0	4 (0.7)
White (includes both European origin and Middle East and North African origin, per NIH definition)	31	361	33	425 (70.6)
More than one racial category	2	7	0	9 (1.5)
Unknown or not Reported	33	14	32	79 (13.1)
Total n (%)	71 (11.8)	457 (75.9)	74 (12.3)	602

Table 2.

Rationale for diagnosis of 8 individuals with variant(s) of unknown significance (VUS) determined to be causative of epilepsy.

ID	Gene	Variant	ACMG Classif.	Zygosity	Inheritance	Rationale for Diagnosis
B0257	*ALG11*	c.1402C>T, p.Arg468Cys	VUS	Hom	Both maternally and paternally inherited	• Fits expected phenotype with infantile-onset intractable epilepsy, spastic quadriplegia and global developmental delay/intellectual disability. • Sister has the same phenotype and homozygous variant. Parents are carriers.
B0272	*GABRG2*	c.1000G>A, p.Ala334Thr	VUS	Het	Maternally inherited	• Phenotype is consistent with the diagnosis: onset at 9 months with refractory generalized epilepsy (absence, rare GTCs) and later developmental delays. • Maternally inherited. Mother also had a similar phenotype with infantile-onset epilepsy and learning disabilities. • ACMG criteria are heavily weighted by *de novo* status but this is a situation of familial disease.
B0542	*NHLRC1* *NHLRC1*	c.656G>A, p.Trp219* c.451G>T, p.Val151Phe	LPATH VUS	CH	Unknown	• Diagnosed with Lafora disease • Skin biopsy with electron microscopy confirmed the presence of Lafora bodies
B0046	*NRXN1* *NRXN2*	c.2686C>T, p.Arg896Trp c.3176G>A, p.Arg1059Gln	LPATH VUS	CH	Maternally inherited Paternally inherited	• Rochtus et al., 2019 describe this patient: “Mutations in *NRXN1* and *NRXN2* in a patient with early-onset epileptic encephalopathy and respiratory depression”.[3] Clinical presentation and known interaction between the *NRXN1* and *NRXN2* proteins lead us to hypothesize that digenic variants in *NRXN1* and *NRXN2* contributed to the phenotype of EIEE, arcuate nucleus hypoplasia, respiratory failure, and death.
B0625	*POLG* *POLG*	c.2243G>C, p.Trp748Ser c.3356T>C, p.Leu1119Pro	PATH VUS	CH	Maternally inherited Unknown	• This patient has a specific EEG pattern, rhythmic high amplitude delta with superimposed polyspikes (RHADs), that is typical for *POLG*-related epilepsy
B0069	*SCN1A*	c.986G>T, p.Gly329Val	VUS	Het	Unknown	• Phenotype is highly suggestive of *SCN1A*-related epilepsy including GTC and myoclonic seizures onset at 10 months, fever and heat sensitivity and status epilepticus with cognitive and behavioral difficulties noted after epilepsy diagnosis. • There are other missense variants at this position have been reported in HGMD. • There are two entries in ClinVar for this variant, one classified as likely pathogenic and one as pathogenic.
B0225	*SCN1A*	c.418A>G, p.Thr140Ala	VUS	Het	Unknown	• The treating epileptologist diagnosed *SCN1A*-related epilepsy.

ID	Gene	Variant	ACMG Classif.	Zygosity	Inheritance	Rationale for Diagnosis
						• Phenotype: refractory focal and secondarily generalized seizures and drop attacks, with early speech delay. • Favorable response to initiation of valproate and reduction of lamotrigine. • Parental testing was not done, which is why it is still classified as a VUS by ACMG criteria.
B0101	*SLC12A5*	c.983A>G, p.Asn328Ser	VUS	Hom	Both maternally and paternally inherited	• Phenotype of Epilepsy of Infancy with Migrating Focal Seizures (EIMFS) as in this individual is highly linked to this genetic disorder, and this individual is included in the literature. • The variant is absent from control populations.[4]

Abbreviations: ACMG = American College of Medical Genetics, CH = compound heterozygous, EEG = Electroencephalogram, EIEE = Early Infantile Epileptic Encephalopathy, GTC = generalized tonic clonic seizures, Het = Heterozygous, HGMD = Human Gene Mutation Database, Hom = Homozygous, LPATH = Likely pathogenic, PATH = pathogenic, VUS = variant of uncertain significance.

Table 3.

Diagnostic yield by phenotypic features. Frequency of key phenotypic features in relation to yield of epilepsy panel and exome testing (n=602, columns 3–4) and to yield of exome after a non-diagnostic epilepsy panel (n=183, columns 5–6).

Phenotype	**Phenotypic categories**	**Yield diagnostic panel +/− exome n (row%)**	**P value, Chi-square or Fisher's exact**	**Yield diagnostic exome after non-diagnostic panel n (row%)**	**P value, Chi-square or Fisher's exact**
Total cohort		n=602[$], diagnostic in 152		n=183[$$], diagnostic in 54	
Sex	Female Male	71 (25.5) 81 (25)	0.8793	27 (29.7) 27 (29.4)	0.9619
ASD (if ≥3y)	Yes No	25 (22.9) 83 (21.9)	0.8184	14 (28.6) 27 (25.2)	0.6602
ID (if ≥5y)	Yes No	52 (25.9) 14 (8.3)	<0.0001 *	25 (34.3) 0	<0.0001 **
Developmental delay (if ≥ 2y)	Global delay Delay in one area None	105 (31.7) 15 (20.8) 13 (8.8)	<0.0001	45 (38.1) 3 (13.6) 0	<0.0001
Developmental regression (if ≥ 3y)	Yes, with epileptic encephalopathy Yes, independent of seizures/change in EEG Yes, unknown or other setting No	23 (22.6) 7 (36.8) 4 (19.1) 75 (21.4)	0.4455	12 (27.3) 3 (33.3) 3 (30) 23 (24.5)	0.8691
Head size	Microcephaly Macrocephaly Normal	26 (30.6) 6 (19.4) 118 (25.2)	0.4085	15 (46.9) 4 (36.4) 35 (25)	0.0427
Systemic malformations	Yes No	13 (33.3) 139 (24.7)	0.2295	8 (47.1) 46 (27.7)	0.0957
Movement Disorder	Yes No	29 (31.5) 122 (24)	0.1269	14 (43.8) 40 (26.5)	0.0518
DEE	Yes No	96 (30.2) 56 (19.9)	0.0037	43 (37.1) 11 (16.4)	0.0032
Dysmorphic features	Yes No	27 (32.5) 125 (24.2)	0.1044	16 (59.3) 38 (24.5)	0.0003
Abnormal metabolic result	Yes No	22 (42.3) 129 (23.8)	0.0033	12 (60) 41 (25.6)	0.0015
CVI (if ≥ 2y)	Yes No	31 (34.1) 96 (21.7)	0.0114	14 (35.9) 32 (24.8)	0.1735
Muscle tone	Abnormal Normal	99 (34.7) 53 (16.7)	<0.0001	46 (43) 8 (10.5)	<0.0001
Epilepsy age of onset ≤ 2y	Yes No	130 (33.3) 22 (10.5)	0.0001	48 (37.8) 6 (10.7)	0.0002
Epilepsy type	Focal Generalized Mixed	54 (27.6) 35 (17.8) 55 (32.7)	0.0048	14 (25.5) 15 (25.4) 19 (34.6)	0.6501
Family history	Consanguinity Epilepsy, first degree relative Notable extended FH of epilepsy	13 (23.6) 20 (29.4) 34 (23)	0.5155[#] 0.4219 0.5920	8 (44.4) 8 (34.8) 9 (20.5)	0.2772[##] 0.6055 0.1354

DEE = Developmental and epileptic encephalopathy; Y = year(s). Features with missing data >10% noted:

[*] 12% missing

[**] 14% missing

[#] 21% missing

[##] 16% missing

[$] Numbers for cohorts with age cutoffs: ≥ 2 years = 544, ≥ 3 years = 498, ≥5 years = 418

[$$] Numbers for cohorts with age cutoffs: ≥ 2 years = 171, ≥ 3 years = 158, ≥5 years = 140

Table 4.

Impact of genetic diagnosis on individual medical management for 152 individuals with infantile or childhood-onset epilepsy

Type of impact on medical management	**n (%) of 152 individuals with genetic diagnosis**	**Case examples**
Impact in any category	110 (72.4)	
Impact in more than one category	51 (33.6)	
Treatment impact	69 (45.4)	
Choice of anti-seizure medications	54 (35.5)	• Treatment with lacosamide (sodium channel blocker) in an individual with a gain-of-function *SCN8A* variant • Treatment with oxcarbazepine in an individual with a *PRRT2* variant with benign familial infantile seizures, with excellent response • Avoidance of sodium channel blockers in an individual with a loss-of-function *SCN2A* variant
Vitamin or metabolic treatments, gene-specific (including ketogenic diet)	10 (6.6)	• Treatment with pyridoxal-5'-phosphate in an individual with a homozygous *PNPO* variant • Treatment with ketogenic diet in individual with glucose transporter disorder (an *SCL2A1* variant) • Treatment with a mitochondrial cocktail in an individual with *POLG* variants
Pathway-driven off-label use of medications	5 (3.3)	• Treatment with memantine in an individual with a *GRIN2A* gain-of-function variant[7] • Discussion of treatment with quinidine in an individual with a *KCNT1* variant • Treatment with riluzole in an individual with an *SCN2A* gain-of-function variant
Disease/gene-specific clinical trials or investigational new drug (IND) use	15 (9.9)	• Consideration of enrollment in ganaxolone clinical trial for an individual with a *PCDH19* variant and another individual with a *CDKL5* variant • Enrollment in a fenfluramine trial for an individual with an *SCN1A* variant
Care coordination (Medical management and monitoring for disease-associated features)	73 (48.0)	• Request for renal ultrasound for an individual with Koolen-De Vries syndrome (*KANSL1* variant) • Referral to multiple specialists for an individual with Mowat-Wilson syndrome (*ZEB2* variant) • Request for EKG and cardiology evaluation for individual with an *SCN1B* variant
Change in prognosis	42 (27.6)	• Counseling on risk of early lethality in an individual with a *BRAT1* variant[5] • Discussion of benign prognosis with future possibility of seizure freedom in an individual with a *PRRT2* variant • Counseling on prognosis in an individual with *NHLRC1* compound heterozygous variant-related Lafora disease
Correction of diagnosis, for those with a diagnosis prior to genetic testing	2 (1.3)	• Clarification of diagnosis for an individual with a *CACNA1A* variant and another individual with a *GNAO1* variant, both previously considered to have primary mitochondrial disorders

Table 5.

Predictors of genetic diagnosis by panel or exome. Multivariate logistic regression analysis for phenotypic predictors of A) diagnostic epilepsy gene panel or exome sequencing and B) diagnostic compared to non-diagnostic exome sequencing after a non-diagnostic epilepsy gene panel. Variables with p-value of <0.25 on univariate testing were initially included in the model, then backwards selection was used to reach the final model with variables having p <0.05 and age of seizure onset ≤ 2 years as a required variable.

Phenotypic predictor variable	Odds ratio (95% confidence interval) for relationship with diagnostic panel or exome	P-value, Wald test
A. Multivariate logistic regression model for predictors of diagnostic epilepsy gene panel or exome, combined		
Age of epilepsy onset ≤ 2 years	3.82 (2.19, 6.66)	<0.0001
Malformation of brain development (Y/N)	0.27 (0.15, 0.51)	<0.0001
Seizure types		
Generalized motor	1.02 (0.65, 1.60)	0.93
Generalized non-motor	1.11 (0.62, 2.01)	0.73
Focal motor	2.29 (1.43, 3.65)	0.0005
Focal non-motor	1.15 (0.73, 1.83)	0.55
Developmental delay, global or in one area	2.64 (1.46, 4.73)	0.0012
B. Multivariate logistic regression model for predictors of diagnostic exome after a negative epilepsy gene panel		
Age of epilepsy onset ≤ 2	2.23 (0.81, 6.19)	0.12 (required in the model)
Tone	3.02 (1.20, 7.59)	0.002
GDD	4.45 (1.14, 17.31)	0.03