Genetic Testing in Children with Epilepsy: Report of a Single-Center Experience

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ABSTRACT: *Background:* Retrospective observational study to determine diagnostic yield and utility of genetic testing in children with epilepsy attending the Epilepsy Clinic at Children's Hospital, London, Ontario, Canada. *Methods:* Children (birth–18 years) with epilepsy, who were seen in a 10-year period (January 1, 2008–March 31, 2018), were selected using defined inclusion criteria and by combining clinic datasets and laboratory records. *Results:* In total, 105 children (52.38% male and 47.61% female) with a variety of seizures were included in the analysis. Developmental delay was documented in the majority (83; 79.04%). Overall, a genetic diagnosis was established in 24 (22.85%) children. The diagnostic yield was highest for whole-exome sequencing (WES), at 35.71%. The yield from microarray was 8.33%. Yields of single-gene testing (18.60%) and targeted multigene panel testing (19.23%) were very similar. Several likely pathogenic and pathogenic variants not previously reported were identified and categorized using ACMG criteria. All diagnosed patients underwent a review of anti-seizure medication management and received counseling on natural history of their disease, possible complications, recurrence risks, and possibilities of preimplantation or prenatal genetic diagnosis. *Conclusions:* Our study confirms the multiple benefits of detecting a genetic etiology in children with epilepsy. Similar yields in single versus multigene testing underscore the importance of accurate clinical phenotyping. Patients with epilepsy and their caregivers in Ontario would undoubtedly benefit from repatriation of multigene panels and WES to the province.

RÉSUMÉ : **Le dépistage génétique chez des enfants atteints d'épilepsie : une étude menée dans un établissement hospitalier.** *Contexte :* Il s'agit d'une étude observationnelle rétrospective visant à déterminer l'efficacité diagnostique et l'utilité du dépistage génétique chez des enfants atteints d'épilepsie qui ont fréquenté la clinique d'épilepsie du *Children's Hospital* à London en Ontario (Canada). *Méthodes :* Ont été sélectionnés des enfants âgés entre 0 et 18 ans qui ont été vus au cours d'une période de 10 ans, soit du 1er janvier 2008 au 31 mars 2018, et qui sont atteints d'épilepsie. Pour ce faire, nous avons, outre l'agencement de données cliniques et de dossiers de laboratoire, défini une série de critères d'inclusion. *Résultats :* Au total, 105 enfants (52,38 % de sexe masculin, 47,61 % de sexe féminin) présentant une variété de troubles convulsifs ont été inclus dans cette étude. Des retards de développement ont été documentés chez la majorité d'entre eux (n = 83), soit 79,04 %. Dans l'ensemble, un diagnostic d'origine génétique a été établi dans le cas de 24 enfants, ce qui représente 22,85 %. L'efficacité diagnostique s'est révélée la plus élevée en ce qui concerne le séquençage de la totalité de l'exome (*whole-exome sequencing*) (35,71 %). En ce qui regarde les autres techniques de dépistage, nous avons obtenu les taux d'efficacité suivants : puces à ADN (*microarrays*) 8,33 % ; dépistage au moyen d'un gène unique 18,60 % ; panel multigénique ciblé 19,23 %. Comme on peut le constater, ces deux dernières techniques ont montré des taux d'efficacité très similaires. De plus, de nombreuses variantes pathogènes et probablement pathogènes n'ayant pas été signalées précédemment ont été identifiées et catégorisées au moyen des lignes directrices de l'*American College of Medical Genetics and Genomics* (ACMG). Tous les patients diagnostiqués ont également fait l'objet d'une révision de la prise en charge thérapeutique de leurs troubles convulsifs et ont bénéficié de conseils au sujet de l'évolution naturelle de leur maladie, de la possibilité de complications, des risques de récurrence et des possibilités d'un dépistage génétique prénatal ou préimplantatoire. *Conclusions :* Notre étude confirme les bénéfices multiples liés au dépistage d'une étiologie génétique chez des enfants atteints d'épilepsie. Des taux d'efficacité similaires tant en ce qui concerne le dépistage monogénique que multigénique mettent en relief l'importance d'un phénotypage clinique précis. Les patients ontariens atteints d'épilepsie et leurs aidants naturels bénéficieraient à n'en point douter du rapatriement des techniques de panel multigénique et de séquençage de la totalité de l'exome dans la province.

RATIONALE

Epilepsy is a chronic neurological condition characterized by an enduring predisposition for unprovoked and recurrent seizures. A seizure is a paroxysmal and transient event associated with the sequential progression of signs and symptoms generated as a consequence of hypersynchronous neuronal firing in the

brain.[1] The prevalence of epilepsy changes with age. In childhood, it has been estimated to affect 4–5 per 1000 children between birth and 15 years of age in population-based surveys.[2] While the precise contribution of genetic etiologies to epilepsy remains unknown, it is estimated that in about two-thirds of persons with epilepsy, there may be an inherited component.[3] Within the subgroup of epileptic encephalopathies undergoing diagnostic exome sequencing, a genetic basis has been confirmed in a proportion as high as 43.3%.[4]

Identification of a genetic basis for childhood epilepsy is gaining importance. A number of recurrent chromosomal copy number variants (CNVs) have been associated with higher seizure susceptibility and variability within families, while other specific *de novo* CNVs have been found to be causal in patients with epilepsy, recognizable dysmorphisms, and developmental delay.[5,6] In terms of single-gene variants as direct causes of epilepsy and comorbid conditions, more than 1000 genes are presently identified, with a dozen frequent players. The list of inherited or sporadic gene defects associated with epilepsy is numerous with significant phenotypic overlap; these include but are not limited to structural causes, channel defects, neurotransmitter impairment, inborn errors of metabolism, and multisystem syndromes.[4,6,7]

There are obvious benefits to establishing a genetic basis. With a specific molecular diagnosis, the family can move on to learning about the disease, its comorbidities, and prognostic implications.[8] Diagnosis often informs management, even in the absence of curative or disease-specific therapy.[9] A timely etiological diagnosis allows for better management, such as influencing choice of anti-seizure medication (ASM), initiation of targeted metabolic and/or dietary treatment, improved surveillance for comorbidities, ability to provide accurate genetic counseling regarding recurrence risks in the family, provision of "closure", and access to specific support groups for families.[10]

Genetic testing for epilepsy in Ontario is in transition. There is currently no next-generation sequencing (NGS)-based multigene panel available for epilepsy as a licensed clinical diagnostic test in Ontario, Canada. Testing is presently accessible through commercial US-based laboratories and paid for by the Ministry of Health and Long-Term Care of Ontario (MOHLTC). The Genetic Testing Advisory Committee was established in Ontario to review the clinical utility and validity of genetic tests and the provision of genetic testing in Ontario. As part of their mandate, the committee also developed recommendations and criteria for genetic testing in epilepsy. These include mandatory prerequisites such as an epileptologist/medical geneticist/clinical biochemical geneticist consultation, a list of diagnostic procedures to be undertaken before genetic testing, criteria for circumstances in which genetic testing is indicated and not indicated, and guidance for selection of genetic tests, including their limitations and considerations.[11] In 2018, an expert Working Group was formed by the Laboratories and Genetics Branch of the MOHLTC of Ontario which included medical geneticists, pediatric neurologists/epileptologists, biochemical geneticists, and clinical molecular geneticists from Ontario to develop a programmatic approach to implementing epilepsy panel testing as a provincial service.[12]

The goal of this study is to collate objective evidence of the current state of the utility of genetic testing technologies in clinical practice at our center. We have completed a retrospective observational study of 105 children attending a tertiary care epilepsy program based in London, Ontario. Our findings represent an overview of the changing landscape of genetic testing and replicate the previous evidence of the utility of integrating NGS-based technologies into the diagnostic pathway of children with epilepsy in a representative population in Ontario.[9,13] Our results further underline the continuing importance of detailed phenotyping and careful selection of genetic testing modality.

METHODS

A retrospective chart review of infants and children from birth to 18 years of age, with a clinical diagnosis of epilepsy, seen in the Epilepsy Clinic of Children's Hospital, London Health Science Centre, from January 1, 2008 to March 31, 2018, was performed to determine diagnostic yield of genetic testing technologies currently accessible in an academic clinical practice setting. Inclusion and exclusion criteria are listed below

Inclusion criteria:

1) A clinical diagnosis of epilepsy meeting current ILAE definition of epilepsy who have undergone any form of cytogenetic and molecular genetic testing
2) Genetic testing results (positive, negative, and equivocal) available.

Exclusion criteria:

2) A prior established diagnosis of a genetic syndrome associated with epileptic seizures as a major clinical presentation.

This project was reviewed and approved by Western University Health Sciences Research Ethics Board (approval number Project ID 111378).

The approach toward utilization of genetic tests in patients with epilepsy at our center has changed over this period. Pediatric neurologists in our division use chromosomal microarray (CMA) as a first-line test in patients selected for genetic testing in patients with epilepsy with or without developmental delay. If the microarray is negative, or a distinctive epilepsy phenotype is noted (e.g. Dravet syndrome, Generalized Epilepsy with Febrile Seizures Plus), then a single-gene targeted testing was often chosen in the initial years. Since gene panel testing was not universally accessible in our province, evaluation often included a genetics consultation. The clinical geneticist would in these cases decide to either use a targeted gene panel or a single gene depending on the epilepsy phenotype. Whole-exome sequencing (WES) was carried out in a very selected group of individuals who met the provincial criteria, in the last few years of the study period, when other tests carried out had not provided a diagnostic result.

We carried out a search through laboratory records in our cytogenetic and molecular laboratories based at the London Health Sciences Centre for relevant genetic tests performed in children with epilepsy. We also obtained a list of patients whose DNA had been sent out of country for molecular genetic testing to commercial laboratories (mostly based in the USA, as multigene panels have not yet been repatriated to our province). We simultaneously screened the clinic datasets maintained in the division of pediatric neurology, as well as the local
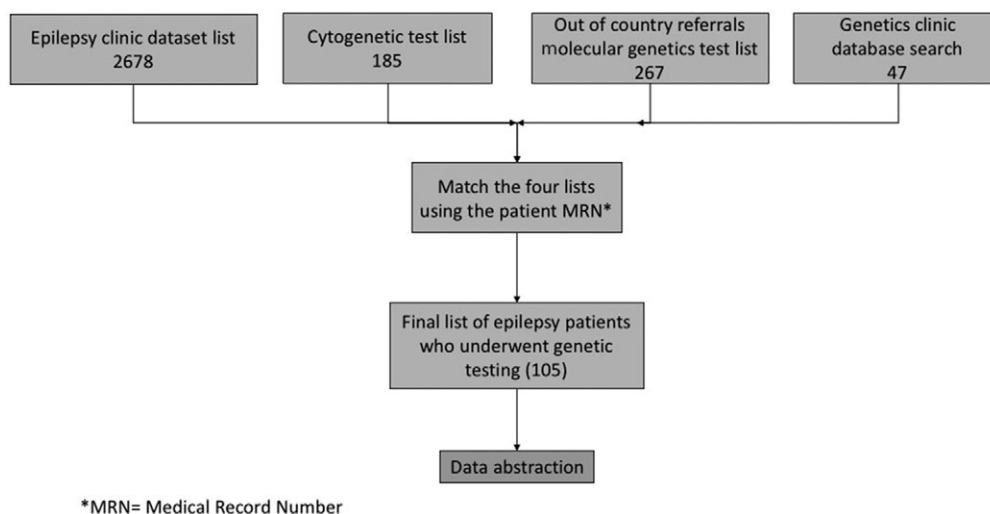
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*MRN= Medical Record Number

***Figure 1**: Flow chart depicting scheme for patient selection for the study population.*

database maintained in the Division of Genetics, for all children with a diagnosis of epilepsy attending outpatient clinics within the time frame described in the inclusion criteria. We then matched the above clinical datasets with the relevant genetic testing to generate a list of patients that met our selection criteria (Figure 1). Data extraction from the clinical and electronic patient chart were carried out to include different variables for each case identified: age, gender, age of onset of epilepsy, seizure type, family history of epilepsy, EEG findings, results of brain imaging studies (CT/MRI), genetic testing, treatments including number of ASMs used, and finally, impact of testing. Variants in genes and chromosomal CNVs were classified using ACMG criteria and variant aggregator datasets such as "ClinVar".[14,15] Diagnostic yield associated with CMA, single-gene testing, multigene panel testing, and WES was calculated as proportion and 95% confidence intervals (CIs) of likely pathogenic and pathogenic variants in the numerator and the number of tests requested (microarray, single-gene testing, targeted multigene panels, and WES) as the denominator. The impact of genetic testing on outcomes was also assessed in descriptive terms. While every effort was made to minimize missing data during data abstraction, data on some variables remained incomplete. Missing data elements for each variable were treated as missing at random during analysis.

We used an automated approach to fitting a logistic regression model so as to identify variables associated with a positive genetic diagnosis on genetic tests (CMA, single-gene panel, multigene manel, or WES). Specifically, we carried out a step-wise selection procedure with the alpha level set at 0.10 for both entry and removal of the candidate independent variables, which included age of onset, seizure type, epileptiform abnormality type, background, number of seizure medications, and presence of developmental delay. In addition, we repeated the stepwise selection procedure while setting the alpha level for both entry and removal of variables at 0.20. For the regression modeling, we used PROC LOGISTIC in SAS v9.4 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

There were a total of 2678 children seen in the epilepsy clinic for "seizures" over the time period of this study. Only 105 (3.92%) of them completed genetic testing, based on our inclusion criteria (Figure 1). Of the 105 children with epilepsy, there were 55 male (52.38%) and 50 female (47.61%), who met the inclusion criteria. Patient demographics and characteristics are summarized in Table 1. The patients were divided into three groups based on their age of onset of seizures as follows: <1 year, 1–5 years, and 6–15 years. Seizure type was identified based on clinical semiology described in the neurology consultation notes in the patient charts. The types of seizures were grouped as focal, generalized, mixed (focal and generalized or multiple seizure types), and non-epileptic (those that were considered as non-epileptic events in the clinic). The majority of seizures at onset were generalized ($n = 63$, 60.57% including two patients with infantile spasms). Thirty-three patients (31.73%) had focal onset seizures, four (3.85%) had mixed seizures, and three (2.88%) had non-epileptic seizures. In one patient, seizure semiology was poorly characterized (0.96%). Developmental delay was noted in 83 (79.04%) of our patients. Of these, motor delay was noted in 40 (48.19%), speech delay in 54 (65.06%), and learning disability in 47 (56.62%). Global delay was described in 29 (34.93%) and autism spectrum disorder in 11 (13.25%) of these patients. Nearly, half of the children, 54 (51.43%) in the study population were enrolled in an individualized educational program at school. The remaining were deemed developmentally normal or were in the preschool age group. Dysmorphic features were identified in 25 (24.04%) cases.

Eight children (7.69%) received no ASMs, 28 (26.67%) were on monotherapy, 26 (24.76%) received two ASMs, while 43 (40.95%) received 3 or more than 3 (maximum 7) ASMs. In terms of seizure control over the 12-month period prior to the last clinic visit, 61 children (58.10%) were noted to have >90% seizure control in comparison to baseline seizure frequency, 11 (10.48%) had moderate control (>50%–90% compared to

Table 1: Patient demographics and characteristics

Demographic/characteristics		Frequency*	Percentage
Age of seizure onset	<1	25	23.81
	1–5	31	29.52
	6–15	19	18.10
	Missing	30	28.57
Sex	Female	50	47.61
	Male	55	52.38
Seizure type	Focal	33	31.73
	Generalized	63	60.57
	Mixed/multiple	4	3.85
	Non-epileptic	3	2.88
	Undetermined	1	0.96
Developmental delay		83	79.04
	Global delay	29/83	34.93
	Motor delay	40/83	48.19
	Speech delay	54/83	65.06
	Intellectual/learning disability	47/83	56.62
	Autism spectrum disorder	11/83	13.25
Dysmorphic/syndromic features	Present	25	24.04
Anti-seizure medications	0	8	7.69
	1	28	26.67
	2	26	24.76
	3+	43	40.95
Seizure control	Poor	32	30.48
	Moderate	11	10.48
	Good	61	58.10
	N/A	1	0.96
School performance	IEP	54	51.43
	Homeschooled	1	0.95
	Mainstream	50	47.62
Number of hospitalizations	0	46	43.81
	1	34	32.38
	2+	25	23.81
	Missing	1	
Number of clinic visits	Range: 1–72	Mean: 12.5	

*Out of $n = 105$, unless otherwise specified.
IEP: individualized education plan.

baseline), and 32 (30.48%) were noted to be poorly controlled (<50% compared to baseline). Only 34 children (32.38%) were hospitalized once, 25 children (23.81%) were hospitalized on at least 2 occasions (range 1–6), and the remaining 46 (43.81%) were never hospitalized.

EEG Findings and Imaging Studies

Ninety-four children had EEG reports available for review; in 81 children (77.88%), the EEG was interpreted as abnormal. Epileptiform abnormalities were documented in 73 (70.9%) of these 81 children, which included focal spikes or spike waves in 25 (34.25%), generalized epileptiform abnormalities in 40 (54.79%), and multifocal epileptiform discharges in 8 children (10.96%). Focal slowing was noted in 5 (6.17%) and 14 (17.28%) records documented generalized slowing of the background rhythms. Magnetic resonance imaging studies of the brain were performed and results were available in 88 children, the majority, and 55 scans (52.38%) were reported as normal, and in 12 records (13.63%) nonspecific changes (either as thinning of the cortical ribbon or the white matter due to ex vacuo change, or nonspecific signal abnormalities in T2-weighted sequences in the white matter) were reported. A specific abnormality was reported in 22 (20.95%). These abnormalities included periventricular nodular heterotopia, subcortical band heterotopia, flattening of the temporal gyri compatible with lissencephaly, agenesis of corpus callosum, multiple subependymal nodules, microencephaly, brain iron accumulation in substantia nigra, Chiari Type I malformation, solitary frontal subependymal heterotopia, multiloculated pineal cyst, and cerebellar atrophy.

Genetic Investigations and Diagnostic Yield

The results of the different genetic test modalities and the respective diagnostic yield are summarized in Table 2. We identified a significant number of novel variants in known epilepsy genes, which are all listed in Tables 3–6 and in Supplementary Table 1.

Chromosomal Microarray (CMA)

CMA was ordered in 84 (80.77%) of 104 patients, the results were normal in 58, variants of uncertain significance (VUS) in 19, likely pathogenic CNV in 4, and pathogenic CNV in 3 patients (Table 3). The diagnostic yield for CMA was estimated to be 8.33% (95% CI 3.41, 16.41).

There were several previously established CNVs detected in our study, such as deletion 2p16.3 involving exons 3–6 of the *NRXN1* (Neurexin-1) gene in a patient with severe intellectual disability and refractory epilepsy; deletion 15q26 involving exons 35–39 of the *CHD2* (Chromodomain Helicase DNA Binding Protein 2) gene in a patient with generalized epilepsy, mild developmental delay, and dysmorphic features, who passed away suddenly at the age of 18 months; and deletion 9q34.11 that removed exons 13–20 of the *STXBP1* (Syntaxin-Binding Protein 1) gene in a patient with global developmental delay, hypotonia, microcephaly, and focal seizures. A microdeletion at 2q23.1 of 0.176 Mb involving exons 1 and 2 of the *MBD5* (Methyl-CpG Binding Domain Protein 5) gene was detected in a patient with global developmental delay, frontal lobe dysplasia, focal frontal lobe epilepsy, and failure to thrive and was reported as likely pathogenic. The microdeletion sizes of this region are variable, and the smallest previously reported microdeletion was approximately 0.038 Mb. Another deletion of 3.78 Mb at 9p24.3p24.2, which is at the extreme distal end of the 9p deletion syndrome region (OMIM# 158170), was also reported as likely pathogenic in a patient with mild developmental delay and febrile seizures (Table 3).

Table 2: Genetic investigations and diagnostic yield

Investigation type	Performed in (out of 105)	Results	Frequency	Diagnostic yield	95% exact confidence intervals
Microarray	84	Normal	59	**8.33%**	(3.41, 16.41)
		VUS	18		
		Likely pathogenic	4		
		Pathogenic	3		
Single-gene testing	43	Normal	33	**18.60%**	(8.39, 33.40)
		VUS	2		
		Likely pathogenic	2		
		Pathogenic	6		
Epilepsy gene panel	26	Normal	11	**19.23%**	(6.55, 39.35)
		VUS*	10		
		Likely pathogenic	2		
		Pathogenic	3		
WES	14	Normal	7	**35.71%**	(12.76, 64.86)
		VUS	2		
		Likely pathogenic	1		
		Pathogenic	4		

*Cases with multiple variants.
VUS: variant of uncertain clinical significance, WES: Whole-exome sequencing.

A novel CNV identified in our study was a large 14.82 Mb duplication at 8q21.13q22.1 interpreted as pathogenic due to its large size and gene content (73 genes, including 37 OMIM genes) in a patient with generalized seizures, speech delay, and subtle dysmorphic features including a prominent forehead and a single palmar crease. The duplication was the product of an unbalanced recombination of an insertion, transmitted from an asymptomatic mother who carried a balanced insertion of the 8q21.13q22.1 segment on her chromosome 14. A similar sized CNV has been reported in three patients with epilepsy, mild developmental delay and epilepsy, supporting the causality in our patient.[16] Cascade testing in this family, therefore, allowed for accurate genetic counseling for the significantly increased recurrence risk.

Single-Gene Testing

This was performed in 43 patients, the results were negative in 33, VUS in 2, likely pathogenic in 2, and pathogenic in 6 patients (Table 4). The diagnostic yield was estimated at 18.60% (95% CI 8.39, 33.40). Among the genes in which pathogenic or likely pathogenic variants were identified by single-gene testing were the *SCN1A* (Sodium Voltage-Gated Channel Alpha subunit 1), *TSC1* (Tuberous sclerosis-1), *TSC2* (Tuberous sclerosis-2), *FLNA,* (Filamin A) and *PTRH2* (Peptidyl-TRNA Hydrolase 2).

Multigene Panels

These were completed in 26 patients (23.08%). Eleven patients had normal results. Seventeen VUS were identified in 10 patients with multiple variants, likely pathogenic variants in 2 patients, and pathogenic variants in 3 patients (Table 5). The diagnostic yield for a targeted epilepsy multigene panel was estimated at 19.23% (95%

CI 6.55, 39.35). The number of genes in multigene panels performed in our patients ranged from 38 to 471.

Among genes in which pathogenic variants were identified by multigene panels were *GABRA1* (Gamma-Aminobutyric Acid Type-A Receptor Subunit Alpha1) (in a 2-year-old patient with Generalized Epilepsy Febrile Seizure plus), *IQSEC2* (IQ Motif and Sec7 Domain ArfGEF 2) (a 2-year-old male patient with X-linked intellectual disability and epileptic encephalopathy), and *STXBP1* (4-year-old boy with Ohtahara syndrome who had infantile spasms since 3 months of age, developmental delay, and brain atrophy). The variants reported as likely pathogenic were a *de novo* variant in the *DCX* (Doublecortin) gene in a patient with extensive band heterotopia and flattening of the lateral temporal gyri compatible with lissencephaly and a *de novo* variant in the *KCNQ2* (Potassium voltage-gated channel subfamily KQT member 2) gene in a patient with epileptic encephalopathy, severe developmental delay, and intractable epilepsy in early life.

Some of the above likely pathogenic and pathogenic variants identified by single-gene testing or multigene panels were novel and have not been previously reported in ClinVar (Tables 4 and 5), except for the *IQSEC2* gene variant that has been previously reported as pathogenic.[5,17]

Whole-Exome Sequencing

Results of WES were available for 14 patients and were normal in 7, VUS reported in 2, likely pathogenic variant in 1, and pathogenic variants in 4 patients, with an estimated diagnostic yield of 35.71% (95% CI 12.76, 64.86) (Tables 2 and 6). The overall diagnostic yield utilizing combining the different genetic test modalities was 23.81% (95% CI 16.04, 33.11).

Table 3: Results of microarray testing

Microarray	Dup/del	Result	Chromosome #	*De novo/* inherited	ClinGen region rating	Elaboration
Likely pathogenic (4)						
	Del 0.176Mb	arr[hg19] 2q23.1(148,746,282-148,922,187)x1	2	N/A	3	Deletion of exons 1 and 2 of the MBD5 gene, associated with developmental delay, autistic features, epilepsy, and dysmorphisms
	Del 0.268Mb	arr[hg19] 2p16.3(50,909,653-51,177,201)x1	2	Paternal	3	Deletion of exons 3–6 of the NRXN1 gene, a candidate gene for susceptibility for schizophrenia and autism spectrum disorder and epileptic encephalopathy. Father is healthy.
	Del 1.448Mb	arr[hg19] 7q35(144,816,917-144,944,315)x1	7	Maternal	0	Deletion includes CNTNAP2 gene associated with autism, dev. delay, and focal seizures. Heterozygous mutations in this gene have been seen in both affected and unaffected individuals. Mother unaffected. Sister with the same deletion is similarly affected and has multifocal seizures and dev. delay.
	Del 3.78Mb	arr[hg19]9p24.3p24.2(163,161-4,036,732)X1	9	Not maternal, father not available	0	Small deletion at the extreme distal end of the 9p syndrome region. Thought to possibly explain dev. delay and hypotonia but not seizures.
Pathogenic (3)						
	Del 0.115Mb	arr[hg19] 15q26.1(93,551,345-93,666,491)x1	15	N/A	3	Deletion associated with epileptic encephalopathy, childhood-onset (OMIM615369), resulted in deletion of exons 35–39 of CHD2 gene.
	Del 0.05Mb	Arr [hg19]9q34.11 (130,435,492-130,485,618)x1	9	N/A	3	Deletion of the exons 13–20 of the STXBP1 gene. Patient diagnosed with early infantile epileptic encephalopathy (OMIM #602926)
	Dup 14.824Mb	arr[hg19] 8q21.13q22.1(82,546,268-97,370,432)x3	8	Result of maternal. Balanced chromosomal translocation	0	Pathogenic, given the size of the duplication. Smaller CNVs have been classified as pathogenic. Thought to explain epilepsy.

CNV: copy number variants

One of the likely pathogenic variants found by WES is in the *SLC2A1* (Solute Carrier Family 2 Member 1), the gene causative of GLUT-1 deficiency. This diagnosis was further confirmed by detecting low glucose levels in the patient's cerebrospinal fluid (CSF Glucose 2.1mmol/l). It also led to diagnostic closure for this family and a switch to the ketogenic diet in terms of management. Finding of a pathogenic variant in the *ANKRD11* (Ankyrin Repeat Domain 11) gene resulted in the diagnosis of KBG syndrome (OMIM #148050) in an adolescent patient without a previous diagnosis. This patient had focal temporal lobe epilepsy, mild intellectual disability, short stature, and dysmorphic features. As expected in KBG syndrome, she grew out of her seizure disorder by the time she reached adolescence. This patient had a family history of Charcot–Marie–Tooth disease and tested positive for a mutation in the GJB1 (Gap Junction Protein Beta 1) gene, and she is currently asymptomatic but will be monitored by a neurologist for the development of symptoms. Finding of a *de novo* variant in the *PACS1* (Phosphofurin Acidic Cluster Sorting Protein 1) gene in a patient with neonatal seizures (subsequently resolved), developmental delay, and mild dysmorphic features resulted in the diagnosis of Schuus-Hoeijmakers syndrome (OMIM #615009).

Another pathogenic variant in the *GABRB3* (Gamma-Aminobutyric Acid Type A Receptor Subunit Beta3) gene was found in a 6-year-old girl with early infantile epileptic encephalopathy, microcephaly, ataxia, developmental delay, and history of developmental regression starting at the age of 14 months. Thus, the finding of variants that closely link with the patients' phenotype and provide biological plausibility for the finding help further patient management by providing a diagnosis.

Impact of Genetic Testing on Patient Outcomes

Based on the results of genetic testing, a change in ASM was made in 4 patients, ketogenic diet was introduced as treatment in 1 patient, and screening for potential complications was implemented in 11 patients. All diagnosed patients received counseling on natural history of their disease, possible complications and

Table 4: Single-gene testing results

Single gene ACMG category	Gene and variant	Hetero/homo or hemizygous	*De novo*/inherited	Mode of inheritance	ClinVar	Diagnosis OMIM number
Likely pathogenic (3)						
	SCN1A: c.3632G > A (p.Cys1211Tyr) Amino acid Cys1211 has been conserved during evolution. *In silico*: probably damaging. Variant resides within the Domain II–III linker region of the protein. Missense mutations of nearby amino acids have been reported in Dravet syndrome.	Het	Mat. Mother has epilepsy	AD	Not listed	SNC1A-related disorder. #604403
	TSC1: c.965dupT (p.Met322Ilefs*19) Patient has met diagnostic criteria for tuberous sclerosis	Het	N/A	AD	Not listed	Tuberous sclerosis. #191100
	SCN1A: c.3733C > T (p.Arg1245Ter) Patient seizure semiology consistent with myoclonic epilepsy	Het	*De novo*	AD	Pathogenic	Myoclonic epilepsy of infancy. #604403
Pathogenic (5)						
	SCN1A: c.2585G > A (p.Arg862Gln) Patient history and seizures consistent with GEFS + phenotype	Het	Parents healthy. Parental testing declined	AD	Not listed	Generalized epilepsy with febrile seizures plus, status epilepticus. #604403
	FLNA: c.5417-1G > C Patient MRI brain confirms periventricular nodular heterotopia	Het	N/A Mother affected	XLD	Not listed	X-linked periventricular nodular heterotopia. #300049
	TSC2:c.894dupT (p.Val299Cysfs*39) Patient has met clinical diagnostic criteria of tuberous sclerosis	Het	*De novo*	AD	Not listed	Tuberous sclerosis. #163254
	PTRH2: c.324G > A (p.Trp108Ter)	Hom	Parents are obligate carriers	AR	Pathogenic	Infantile-onset multisystem neurologic, endocrine, and pancreatic disease. #616263
	PLA2G6 (N/A) Testing done at a different hospital. Report not available.		NA	AR	NA	Neurodegeneration with brain iron accumulation. #610217

VUS: variant of uncertain clinical significance

recommended screening, management, recurrence risks, and possibilities of preimplantation or prenatal genetic diagnosis.

In 24 children, the genetic diagnosis ended a diagnostic odyssey for the parents and permitted diagnostic closure as well as a reduced need for further investigations. Thirty-one family members (majority were parents) underwent genetic testing following the identification of the pathogenic/likely pathogenic mutation. Overall, a positive impact on management was made possible in 17 patients (16.34%) based on genetic testing results. In the remaining eight (7.69%) patients, the genetic test served to confirm the clinical diagnosis that had already been disclosed to the patient (e.g. Tuberous sclerosis, structural brain malformation). The impact of genetic testing has been summarized in Table 7.

Clinical Variables as Predictors of Outcome of Genetic Testing

At $p < 0.05$ level of statistical significance, no single variable emerged as a predictor for the likelihood of a positive genetic diagnosis. With $p < 0.10$ level of statistical significance, the presence of developmental delay was the single variable that was retained in the stepwise model with a point estimate of 0.276

(95% CI 0.077, 0.984). Further relaxation at a $p < 0.20$ level of statistical significance, the presence of developmental delay continued to be the only variable retained in the predictive model (Supplementary Table 2).

DISCUSSION

As it stands, the type and timing of genetic testing in epilepsy in Ontario is largely determined by individual neurologists and/or geneticists caring for these patients, based on different variables under consideration for each physician. The use of genetic testing also depends on access to certain types of tests sent out of province, due to ministry restrictions. To help understand the practice patterns prior to the development and implementation of provincial genetic testing criteria in epilepsy, the goal of this study was to document experience with genetic testing in epilepsy in pediatric tertiary care hospital, based in London, Ontario between 2008 and 2018.[11,12] Our study outlines the utility of all clinically available genetic testing during that time period, including CMA, single-gene testing, targeted multigene panels, and WES, in finding an underlying genetic cause in this population.

Table 5: Gene panel test results

Epilepsy panel or other panel ACMG category	Gene and variant	Hetero/homo or hemizygous	*De novo*/inherited	Mode of inheritance	CLinVar	Diagnosis and OMIM number
Likely pathogenic (3)						
	DCX: c.383C > T (p.Ser128Phe)	N/A	*De novo*	XL	Not listed	X-linked lissencephaly type 1 #300067
	KCNQ2: c.901G > A (p.Gly301Ser) *In silico:* probably damaging	Het	*De novo*	AD	Conflicting interpretation of pathogenicity (pathogenic, likely pathogenic, VUS)	Early infantile epileptic encephalopathy type 7 #613720
	GRIN2A: c.2146G > A (p.Ala716Thr)	Het	*De novo*	AD	Not listed	Epilepsy, focal with speech disorder and with or without mental retardation #245570
Pathogenic (3)	GABRA1: c.641G > A (p.Arg214His) Not previously reported variant. Arg 214 is conserved across species. A different missense change at 214 previously reported in patients with epilepsy. *In silico*: probably damaging.	Het	*De novo*	AD	Pathogenic/Likely pathogenic	Generalized epilepsy with febrile seizures plus GEFSP #615744 While GABRA1 mutations have been reported with an EIEE phenotype, this patient presented with recurrent febrile and afebrile seizures with focal features and absences
	IQSEC2: c.2911C > T (p.Arg971Ter) Nonsense variant predicted to cause loss of protein function either through protein truncation or nonsense-mediated mRNA decay.	Het	*De novo*	AD	Likely pathogenic	X-linked intellectual disability and epileptic encephalopathy #300532
	STXBP1: c.1663G > T (p.Glu555Ter) Previously unpublished nonsense-mutation predicted to cause loss of normal protein function through protein truncation and results in the loss of 48 amino acids.	Het	*De novo*	AD	Not listed	Epileptic encephalopathy, early infantile, 4. Ohtahara syndrome #612164

Our overall rate of a genetic diagnosis of 23.81% is in keeping with published literature, particularly with studies that span several years.[9,18]

Chromosomal Microarray

The diagnostic yield of CMA of 8% is similar to that reported from other published data of approximately 10%,[18,19] and the majority (77%) of patients in whom CMA was requested had developmental delay in addition to epilepsy. Among the pathogenic CNVs identified in our study, there were well-known epilepsy hotspots,[20] as well as previously unreported findings (such as the 8q21.13q22.1 duplication). In keeping with published literature, significant CNVs were detected among our patients with epilepsy accompanying a multisystem syndrome, most often in the setting of developmental delay and dysmorphic features.[19,21]

There were some limitations to the interpretation of CNVs in our epilepsy patients. Firstly, due to the timing of their presentation and variable degrees of genetics follow-up, some of our patients with epilepsy were only offered CMA (and no molecular testing), and we were not able to exclude the contribution of single-gene variants in these patients. There were also instances where familial cascade testing was not possible, due to unavailability of parent/parents, limiting our ability to effectively assess the contribution of particular CNVs, especially the ones which are established to have variable presentations.

Molecular Testing: Single-Gene Testing, Multigene Panels, WES

Recent development of NGS technologies has identified several new genes responsible for monogenic epilepsy with high penetrance with the development of multigene epilepsy

Table 6: Whole-exome sequencing results

WES ACMG category (n)	Gene and variant	Hetero/homo or hemizygous	De novo/inherited	Mode of inheritance	ClinVar	Diagnosis and OMIM number
Likely pathogenic (1)						
	SLC2A1: c.274C > T (p.Arg92Trp) Amino acid position 92 is conserved among species. *In silico*: probably damaging Patient was confirmed to have GLUT-1 deficiency by low glucose levels in CSF, 2.1mmol/l. Clinically presented with episodic paraplegia and developmental delay but no seizures.	Het	N/A, adopted	AD	Pathogenic/likely pathogenic	GLUT-1 deficiency #612126
Pathogenic (4)						
	ANKRD11: c.2143G > T (p.Glu715Ter) Variant not previously reported. Predicted to cause loss of protein function via truncation and nonsense-mediated protein decay. GJB1 c.633C > A (p.Tyr211X)	Het Het	Parental testing declined Positive family history	AD XL	Not listed Pathogenic	KBG syndrome (designation follows Opiotz's practice of using initials of surnames of families affected) Short stature, distinctive craniofacial features, intellectual disability, and seizures #148050 X-linked Charcot–Marie–Tooth disease #302800
	PACS1: c.607C > T (p.Arg203Trp)	Het	*De novo*	AD	Pathogenic	Schuurs-Hoeijmakers syndrome #615009
	GABRB3: c.358G > A (p.Asp120Asn)	Het	*De novo*	AD	Pathogenic	Epileptic encephalopathy early infantile type 43 #617113

panels[18,22,23] designed to maximize the yield. Early integration of WES and targeted multigene panels into the diagnostic pathway has been shown to increase not only diagnostic yield and clinical utility in such cases but also cost-effectiveness.[8,13] Given that our study spanned 10 years prior to 2018, single-gene testing was performed in a considerable number of patients (n = 41, 39.4%), mostly before NGS panels became clinically available. Multigene panel testing was only performed in a minority of our cohort (n = 26, 25%) and even a smaller number of patients (n = 13, 12.5%) had access to WES. This is due to various factors, including the recent clinical availability of either test in Ontario (multigene panels for the latter 5 years and WES for the latter 3 years of this study), the provincial restrictions on out-of-country testing, as well as the change in practice culture among physicians, who likely became increasingly familiar with NGS only toward the end of the last decade.[24] The advantage of testing multiple (mostly hundreds of) genes at the same time over single-gene sequencing is indisputable. However, the overall diagnostic yield of multigene panels (19.23%) is very similar in comparison to the yield of single-gene testing (18.61%) in our cohort. Although our numbers are small, this finding speaks to the importance of accurate phenotyping of seizure semiology, as well as clear delineation of clinical features, to make informed and cost-efficient choices when it comes to genetic testing. Further, genotype–phenotype correlations are improving particularly in the recognition of

early-onset epileptic encephalopathies, where the diagnostic yield is decidedly higher.[25,26]

As expected and shown in multiple studies worldwide, WES had the highest diagnostic yield of 35.71% (Tables 2 and 6) in our pediatric epilepsy cohort.[4,5,9,13,26] In all 14 patients, WES was requested as the last-tier genetic investigation, after CMA, single-gene and/or multigene panel testing failed to reveal a genetic diagnosis. The yield would likely be even higher, or the clinical impact of the results would be stronger, if WES was available to more patients and earlier in the diagnostic odyssey in this population. While we do not have data into "time-to-diagnosis" in our cohort, multiple studies have now shown the benefit of first-tier WES on multiple occasions, especially in the pediatric intensive care setting.[27-30] WES is not currently performed in Ontario as a clinical diagnostic test and requires the Ministry of Health approval to be performed as an out-of-country test, while the technology and expertise already exists in the province. Our results confirm that patients with epilepsy and their caregivers in Ontario would certainly benefit from repatriation of multigene epilepsy panels and WES to our province.

Impact of Genetic Testing

The findings of our study endorse the multiple benefits of detecting an underlying genetic diagnosis in pediatric patients with epilepsy. Overall, 17 (16.34%) patients had a change in their epilepsy management, surveillance, or prognosis based on their

Table 7: Outcomes and impact of genetic testing

Gene	Diagnosis	Positive impact of genetic diagnosis
ANKRD1 and GJB1	KBG syndrome and Charcot–Marie–Tooth disease	Dysmorphic features, intellectual disability, and epilepsy explained. Prognosis for remission of seizures during adolescence disclosed. Patient will be monitored for symptoms of Charcot–Marie–Tooth disease by a neurologist. Family history of Charcot–Marie–Tooth disease is explained and testing offered to extended family.
DCX	DCX-related disorder	Intellectual disability, epilepsy, and subcortical band heterotopia on MRI explained. Prognosis for refractory epilepsy and behavioral problems disclosed. Eye examination requested as a screening for associated complications. *De novo* status reviewed.
FLNA	Periventricular nodular heterotopia	Seizure disorder and periventricular nodular heterotopia explained. Echocardiogram requested to screen for associated complications. Mother, similarly affected, counseled about recurrence risks, possibilities of preimplantation, and prenatal genetic diagnosis.
GABRA1	Infantile epilepsy GEFS+	Epilepsy and developmental delay explained. *De novo* status disclosed.
GABRB3	Epileptic encephalopathy early infantile type 43	Epilepsy, developmental delay, and ataxia explained. *De novo* status reviewed with the parents.
GRIN2A	Landau–Kleffner syndrome/acquired epileptic aphasia	Epilepsy, an episode of total aphasia at the age of 4 years, expressive language dyspraxia, and generalized and focal spikes on EEG during sleep explained. *De novo* status disclosed.
IQSEC2	X-linked intellectual disability and epileptic encephalopathy	Epileptic encephalopathy, developmental delay, and regression explained. *De novo* status disclosed.
KCNQ2	Early-onset infantile epileptic encephalopathy type 7	Intractable epilepsy in early life and severe intellectual disability explained. Medications reviewed and no changes deemed necessary. *De novo* status disclosed.
PACS-1	PACS1-related disorder Schuurs-Hoeijmakers syndrome	Seizures in infancy, dysmorphisms, and intellectual disability explained. Echocardiogram and kidney ultrasound requested as screening for associated congenital anomalies. *De novo* status disclosed.
PTRH2	Infantile-onset multisystem neurologic, endocrine, and pancreatic disease	Severe global developmental delay, insulin-dependent diabetes, and epilepsy explained. Anti-seizure medications reviewed and no change was deemed necessary. Two brothers, similarly affected, are undergoing testing for the familial mutation. Parents were confirmed to be carriers. Recurrence risks and opportunities for preimplantation and prenatal genetic diagnosis reviewed.
SCN1A (three patients)	SCN1A-related disorder	Medications reviewed in all three patients. In two-thirds patients, a change in anti-seizure medications was required. One-third did not require a change in medications. Recurrence risks reviewed. In one-third cases, the mother also had epilepsy and carried the same variant. Opportunities for prenatal diagnosis were discussed.
SLC2A1	GLUT-1 deficiency	Symptoms of episodic paraplegia and developmental delay explained. Ketogenic diet offered.
STXBP1	Epileptic encephalopathy type 4	Infantile spasms and developmental delay explained. Prognosis for severe developmental delay, tremors, and seizures responsive to medications disclosed. *De novo* status disclosed.
TSC1	Tuberous sclerosis	Screening for potential complications initiated, recurrence risks reviewed with the patient, and opportunities for preimplantation and prenatal genetic diagnosis discussed. M-TOR inhibitor eventually started for treatment of renal angiomyolipomas. Family history reviewed, and no other family members are affected.
TSC2	Tuberous sclerosis	MRI brain and Lung CT scan arranged as screening for possible complications. *De novo* status disclosed. Recurrence risks reviewed with the patient, and opportunities for preimplantation and prenatal genetic diagnoses discussed.
	Del 2q23.1	Deletion was thought to explain developmental delay and epilepsy. Parental testing declined.
	Del 2p16.3	Deletion was thought to explain developmental delays and epilepsy. Deletion was found to be inherited from the father who had developmental delays and learning difficulties growing up. Recurrence risks reviewed. Opportunities for preimplantation and prenatal genetic diagnosis discussed. Patient referred to Developmental Pediatrics.
	Del7q35	Deletion was thought to explain focal epilepsy. Deletion was maternally inherited, although the mother was unaffected; similarly affected sister had the same deletion and recurrence risks for future pregnancies were discussed.
	Del 9p24	Deletion was thought to explain developmental concerns. School assessment of learning needs was recommended. Healthy mother tested negative, and father was healthy and was not available for genetic testing.
	Del 15q26	The deletion included CHD2 gene. Prognosis for severe epilepsy refractory to treatment was disclosed. Patient was referred to neurology for an assessment of abnormal movements and treatment and was started on anti-seizure medication. Patient passed away suddenly at the age of 18 months.
	Del 9q34.11	STXBP1 gene involved. Deletion was thought to explain developmental delay, hypotonia, and possible autistic features. Patient referred to neurology and ophthalmology. *De novo* status discussed with the parents.
	Dup 8q21.13q22.1	Duplication was thought to explain dysmorphic features, developmental delay, and seizures. Parental testing revealed maternal balanced chromosomal rearrangement. Sibling was tested and was found to have the same condition. Recurrence risks and opportunities for prenatal and preimplantation genetic diagnosis discussed with the family.

genetic testing results and many families benefited from more specific counseling and increased options. While our numbers are small, the individual impact on each of the individuals and their families is significant and speaks to the benefit of the increasing implementation of genetic testing in epilepsy care in our institution.

Amongst the 2678 children evaluated for epilepsy during the period of the study, there likely were individuals who were not eventually diagnosed with epilepsy, or who had genetic testing performed elsewhere, or who did not meet the criteria for genetic testing in our province, the rate of systematic access to genetic testing was still lower than expected. We hope our findings of the increasing impact of genetic testing in epilepsy will provide further incentive for clinicians to consider these tests earlier in the diagnostic pathway.

Study Limitations

An observational study of this nature carries all the limitations associated with retrospective data collection and analysis. The descriptions of seizure semiology and EEG interpretations relied on the reports of individual physicians. The sample size being small, the resulting diagnostic yields have wide CIs; hence, the reader is advised caution in the interpretation of test results and its application on a wider population basis. It may also explain the limitations of the statistical model in identifying reliable clinical predictors of diagnostic test results.

CONCLUSIONS

This study had the advantage of an established clinical collaboration between epileptologists, geneticists, and laboratory professionals, which enabled deep phenotyping of both epilepsy semiology and non-neurological features, cascade familial testing whenever available to help resolve results, consistent variant interpretation, and comprehensive genetic counseling of patients and families. Being a single-center experience in Ontario was both a strength (real-life example of a particular time frame in Canada depicting temporal trends) and a limitation (small sample size and limited availability of NGS tests) of our study. Despite being able to offer some form of genetic testing to most of our patients with epilepsy, we were not able to show any predictive marker for positive genetic testing results, other than developmental delay, likely due to our sampling size. The revolution and changing trends we witnessed in genetic testing increased our yield and therapeutic success, but our study also highlighted the somewhat arbitrary selection of genetic testing based on physician experience, preference, and access. While some of our conclusions remain speculative, our results confirm the increased need of pediatric epilepsy patients in Ontario for a consistent approach to genetic testing and access to more genome-wide testing in a timely manner.

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DISCLOSURES

The authors have no conflicts of interest to declare.

STATEMENT OF AUTHORSHIP

SL participated in the collection and abstraction of clinical and laboratory data, creation of a dataset, and analysis of results.

NK participated in the intial development of research protocol, ethics approval, variant interpretation, and writing up of the results and discussion about the genetic testing and implications for outcome. She also mentored So Lee through the entire period for the collation of data.

EZA participated in the development of research protocol and ethics approval, created a dataset of epilepsy patients seen in the neurology clinics, and identified clinical variables that were to be included.

PY participated in the development of study protocol, faciliated the search of laboratory data on microarray studies in patients with epilepsy, and reviewed the draft of manuscript.

BS participated in the development of study protocol, faciliated the search of laboratory data on molecular genetic studies in patients with epilepsy, and reviewed the manuscript drafts.

TBB participated in the discussion of results of molecular genetic testing, variant interpretation, and writing up of the discussion in the final drafts of the manuscript.

ANP was the lead in the study design, ethics submission, supervision and mentoring during data collection, writing up of the rationale, methods, results and discussion, and final submission of the paper.

SUPPLEMENTARY MATERIAL

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