Diagnostic Yield and Treatment Impact of Targeted Exome Sequencing in Early-Onset Epilepsy

Michelle Demos[1]*[†], Ilaria Guella[2][†], Conrado DeGuzman[1], Marna B. McKenzie[2], Sarah E. Buerki[1,3], Daniel M. Evans[2], Eric B. Toyota[1], Cyrus Boelman[1], Linda L. Huh[1], Anita Datta[1], Aspasia Michoulas[1], Kathryn Selby[1], Bruce H. Bjornson[1], Gabriella Horvath[4], Elena Lopez-Rangel[5], Clara D. M. van Karnebeek[6,7], Ramona Salvarinova[4], Erin Slade[1], Patrice Eydoux[8,9], Shelin Adam[10], Margot I. Van Allen[10], Tanya N. Nelson[8,9], Corneliu Bolbocean[11,12], Mary B. Connolly[1] and Matthew J. Farrer[2]

[1] Division of Neurology, Department of Pediatrics, University of British Columbia and BC Children's Hospital, Vancouver, BC, Canada, [2] Department of Medical Genetics, Centre for Applied Neurogenetics (CAN), University of British Columbia, Vancouver, BC, Canada, [3] Division of Neuropediatrics, University Children's Hospital Zurich, Zurich, Switzerland, [4] Division of Biochemical Diseases, Department of Pediatrics, BC Children's Hospital, University of British Columbia, Vancouver, BC, Canada, [5] Division of Developmental Pediatrics, Department of Pediatrics, BC Children's Hospital, University of British Columbia, Vancouver, BC, Canada, [6] Department of Pediatrics, Centre for Molecular Medicine and Therapeutics, BCCHRI, University of British Columbia, Vancouver, BC, Canada, [7] Department of Pediatrics, Academic Medical Centre, Amsterdam, Netherlands, [8] Division of Genome Diagnostics, Department of Pathology and Laboratory Medicine, BC Children's Hospital, Vancouver, BC, Canada, [9] Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada, [10] Department of Medical Genetics, BC Children's and BC's Women's Hospitals, University of British Columbia, Vancouver, BC, Canada, [11] University of Tennessee Health Science Center, Memphis, TN, United States, [12] Centre for Addiction and Mental Health, Toronto, ON, Canada

Targeted whole-exome sequencing (WES) is a powerful diagnostic tool for a broad spectrum of heterogeneous neurological disorders. Here, we aim to examine the impact on diagnosis, treatment and cost with early use of targeted WES in early-onset epilepsy. WES was performed on 180 patients with early-onset epilepsy ($\leq$5 years) of unknown cause. Patients were classified as Retrospective (epilepsy diagnosis >6 months) or Prospective (epilepsy diagnosis <6 months). WES was performed on an Ion Proton[TM] and variant reporting was restricted to the sequences of 620 known epilepsy genes. Diagnostic yield and time to diagnosis were calculated. An analysis of cost and impact on treatment was also performed. A molecular diagnoses (pathogenic/likely pathogenic variants) was achieved in 59/180 patients (33%). Clinical management changed following WES findings in 23 of 59 diagnosed patients (39%) or 13% of all patients. A possible diagnosis was identified in 21 additional patients (12%) for whom supporting evidence is pending. Time from epilepsy onset to a genetic diagnosis was faster when WES was performed early in the diagnostic process (mean: 145 days Prospective vs. 2,882 days Retrospective). Costs of prior negative tests averaged $8,344 per patient in the Retrospective group, suggesting savings of $5,110 per patient using WES. These results highlight the diagnostic yield, clinical utility and potential

cost-effectiveness of using targeted WES early in the diagnostic workup of patients with unexplained early-onset epilepsy. The costs and clinical benefits are likely to continue to improve. Advances in precision medicine and further studies regarding impact on long-term clinical outcome will be important.

Keywords: targeted WES, early-onset epilepsy, diagnostic yield, cost estimation, Canada

INTRODUCTION

Epilepsy is a common pediatric neurological disorder associated with an increased risk of developmental delay, autism and psychiatric illness; and for which treatment is ineffective in 30–40% of patients. High-throughput sequencing (HTS) has become a widespread diagnostic tool in various genetic conditions, including epilepsy (1), vastly improving molecular diagnosis. Its clinical utility has been proven in epileptic encephalopathies and in mixed epilepsy cohorts (2–11); and in neurodevelopmental disorders (12–14) in which epilepsy is a comorbid feature. The diagnostic yield ranges between 10 and 60%, settling around 25% in large studies with broader inclusion criteria, and with comparable diagnostic rates between gene panels and WES (1). A genetic diagnosis of epilepsy may enable more accurate counseling regarding prognosis and recurrence risk, avoids unnecessary medical investigations and may change care. It also allows families to connect with the same genetic condition and/or join support groups. Recent studies have demonstrated the potential cost savings of WES in the diagnostic work-up of children with suspected monogenic disorders (15–22). However, in Canada, access to such technology in clinical care is variable. In this British Columbia study, we assess the effectiveness of using WES by comparing diagnostic yield, time to diagnosis, and cost to current clinical practices. The potential treatment impact of a genetic diagnosis is also described.

METHODS

Patients

One-hundred and eighty patients with epilepsy (23) were enrolled between December 2014 and September 2018. All had seizure onset at ≤5 years of undefined cause after clinical evaluation, EEG, brain MRI and chromosome microarray investigations. Seizure types and electroclinical syndromes were classified according to the International League Against Epilepsy (ILAE) (24). Patients with self-limiting benign electroclinical syndromes, such as Childhood Absence Epilepsy (onset >4 years), were excluded as they most likely have multifactorial inheritance. Patients were classified as **Retrospective** ($n = 127$), defined as an epilepsy diagnosis >6 months before study enrollment with a standard clinical approach to genetic testing (variable genetic tests which include gene-by-gene approach using Sanger sequencing, small epilepsy gene panels using high-throughput sequencing, and/or mitochondrial DNA sequencing; or **Prospective** ($n = 53$), which included an epilepsy diagnosis <6 months before study enrollment date and having limited to

no genetic testing. Varying degrees of screening tests for inborn errors of metabolism; such as plasma amino acids, lactate and ammonia, were also performed in both groups. Clinical data was recorded using a secure Research Electronic Data Capture (REDCap) (25) information system hosted at the Child and Family Research Institute.

This study was approved by the BC Children's Hospital and University of British Columbia Ethics Board (protocol number H14-01531). Informed consent and/or assent were obtained before study inclusion in accordance with the Declaration of Helsinki.

Whole-Exome Sequencing

Genomic DNA was extracted from peripheral blood lymphocytes following standard protocols. Exonic regions were captured using the Ion AmpliSeq Exome Kit (57.7 Mb) and WES was performed on an Ion Proton™ according to manufacturers' recommendations (Life Technologies Inc., CA) within 2 weeks of receiving samples. Reads were aligned against the human reference genome hg19. Variant annotation was performed with ANNOVAR (26) integrating data from PHAST PhyloP (27), SIFT (28), Polyphen2 (29), LRT (30), and MutationTaster (31) algorithms, Combined Annotation Dependent Depletion (CADD) scores (32), dbSNP (www.ncbi.nlm.nih.gov/SNP/), the Genome Aggregation Database (gnomAD; gnomad.broadinstitute.org) and ClinVar (33) (www.ncbi.nlm.nih.gov/clinvar). Additionally, variants were compared to an in-house database containing more than 900 exomes to exclude platform artifacts and common variants not present in public databases.

Analysis was restricted to 620 genes previously implicated in epilepsy (**Supplementary Table 1**), using a gene-reporting pipeline developed in-house. The gene list was compiled through the combination of a comprehensive literature search (Pubmed, OMIM) and clinically available epilepsy panels. Annotation was limited to exonic non-synonymous and splicing (±5 bp) substitutions. Homozygous variants, potential compound heterozygous variants (defined as genes with >1 variant locus per individual) with a minor allele frequency (MAF) <1% and heterozygous variants with MAF <0.01% were reported. All samples were required to meet minimum quality standards, with a WES average coverage >80X.

Sanger sequencing, performed as previously described (34), was used on a case-specific basis in a few individuals with very specific clinical phenotypes to complete regions of poor coverage in genes related to the patient's phenotype when no candidate variants were identified, or when a heterozygous and potentially pathogenic variant was identified in gene previously

implicated in autosomal recessive disease. No additional variants were identified though post-WES Sanger sequencing.

Variant Prioritization and Validation

Cases were reviewed at a bi-weekly meeting by a multi-disciplinary genomic team. Variant prioritization was performed based on: (1) frequency in public databases; (2) predicted protein impact; (3) disease inheritance, and; (4) correlation of patient phenotype and candidate gene literature. Up to 3 putative causative variants were validated by Sanger sequencing in patient and parental samples. Clinical Sanger sequencing confirmation and interpretation in accordance with ACMG guidelines (35) allowed disclosure to families and management adjustments when indicated. Two time intervals were measured for the first 50 patients: (1) from a clinical diagnosis of epilepsy to Sanger validation of a putative pathogenic variant; and (2) from enrollment with genetic counseling to Sanger validation of the same.

Additional Analysis

Exome data was periodically reanalyzed in patients, if no genetic diagnosis was identified in the initial analysis. Moreover, trio WES was performed in 27 patients (22 Retrospective and 5 Prospective) with no diagnosis.

Genetic Counseling and Treatment Implications

Pre- and post-test genetic counseling was performed for each patient/family. As only a limited set of 620 genes related to seizure disorders were annotated, and only in affected probands, reporting related to incidental (secondary) findings was uncommon (36). Genetic disorders with specific therapeutic implications (47 genes) were defined as conditions in which current literature supports a preferred antiepileptic medication and/or approach (37–39).

Cost Estimation

For the first 50 patients, resource use data were retrospectively acquired from electronic health records and medical charts. Cost estimates in Canadian dollars were based on micro-cost information from the British Columbia Provincial Medical Service Plan Index (2015), Canadian Interprovincial Reciprocal Billing Rates (2014/2015), Children's and Women's Health Center of British Columbia Internal Fee Schedule (2015) and the internal accounting system. Diagnostic costs included: biochemical tests, imaging tests, genetic tests, neurophysiological tests, and biopsies (a complete list of tests is provided in **Appendix 1**). Academic and/or hospital pricing is used throughout. Inpatient hospitalization costs, outpatient visits such as clinic visits, and indirect costs such as parental time off work for medical visits related to their child's epilepsy were not included. All categorical and quantitative variables were analyzed using STATA (Release 13, College Station, TX).

TABLE 1 | Results: Demographics and diagnostic yield.

	All Patients (N = 180)	Prospective (N = 53)	Retrospective (N = 127)
Age at Epilepsy Onset (months) average (range)	18 (0.03–60)	16 (0.1–60)	18 (0.03–60)
Males; Females	77;103	27;26	50;77
DIAGNOSIS			
Definite/likely	59 (33%)	21 (40%)	38 (30%)
Treatment Implications	**27 (46%)**	**15 (71%)**	**12 (32%)**
Possible	21 (12%)	4 (8%)	17 (13%)

Patients highlighted in bold are those for whom a genetic diagnosis had treatment implications.

RESULTS

Targeted WES was performed on 180 subjects and clinical features are summarized (**Supplementary Table 2**-first 50 patients are indicated in bold). Detailed clinical information has previously been reported for subjects 002, 013, 044, 073, and 144 (40–43). The average age of epilepsy onset was 18 months (range 0.03–60 months), 16 months for Prospective cases (n = 53) and 18 months for Retrospective cases (n = 127) (**Tables 1**, **2**). Of the 620 genes, 87% had at least 80% of their consensus-coding region sequenced with >20X coverage (**Supplementary Table 1**).

Diagnostic Yield

A molecular diagnosis was established in 59/180 patients (33%) (**Table 1**). Pathogenic/likely pathogenic variants were identified in 41 genes. The majority of the diagnosed patients (41/59) had an autosomal-dominant disorder; of these 33 had a *de-novo* variant and remaining inherited from an affected parent and/or inheritance status unknown for one or more parent. Five patients had an autosomal-recessive disorder and the remaining 13 patients had an X-linked disorder (10 patients with X-linked dominant variants and 3 male patients with maternally inherited X-linked recessive disorders). In addition, a variant of uncertain significance (VUS) possibly explaining the clinical symptoms of the index patient was identified in 21 cases (12%) (**Supplementary Table 3**).

The diagnostic yield was higher in the Prospective (40%) than Retrospective group (30%). Patients in whom a diagnosis was made had earlier onset epilepsy (mean 13.2 vs. 21.9 months), and an epileptic encephalopathy was more common (**Table 3**). Of 82 patients with epileptic encephalopathy a definite or likely pathogenic variant was identified in 36 (44%) (**Supplementary Table 4**).

Treatment Implications

A genetic disorder with specific therapeutic implications was diagnosed in 27 patients and management was influenced and/or altered in 23 (12 Prospective and 11 Retrospective). Clinical information, treatment changes, and impact are summarized (**Table 2**).

TABLE 2 | Patients with definite/likely diagnosis and treatment impact.

ID Sex	AAO	Epilepsy type	Gene (IP)	GRCh37/hg19	Variant	Zyg	Origin	Treatment Impact
PROSPECTIVE PATIENTS								
001 M	3 m	Dravet	SCN1A (AD)	chr2:166848491	NM_001165963:c.5294T>C;p.F1765S	het	De novo	Change from levetiracetam to clobazam, valproic acid, and topiramate
010 F	18.3 m	Unclassified	SMC1A (XLD)	chrX:53409269	NM_006306:c.3321C>G;p.Y1107X	het	De novo	
018 F	6 m	Ohtahara, West	STXBP1 (AD)	chr9:130434396	NM_003165:IVS12+1GT>AA;NA[c]	het	De novo	
033 F	9.2 m	EE	POLG (AR)	chr15:89871929	NM_002693:c.1157G>C;p.R386P	comp het	♀carrier	Stopped valproic acid; early palliative care; prenatal testing for next pregnancy
				chr15:89866657	NM_002693:c.2243G>C;p.W748S		♂carrier	
059 M	24 m	Unclassified	GABRA1 (AD)	chr5:161322788	NM_000806:c.973T>C;p.F325L	het	De novo	
069 M	51.5 m	Unclassified	MED23 (AR)	chr6:131944505	NM_015979:c.382G>A;p.G128R	comp het	♀carrier	No change in management
				chr6:131941826	NM_015979:c.539C>A;p.A180D		♂carrier	
098 M	1.5 m	Unclassified	KCNT1 (AD)	chr9:138660693	NM_020822:c.1420C>G;p.R474G	het	De novo	Stopped phenobarbital at 2 m; avoided MRI with anesthetic; seizure free and normal development at 6 m
104 M	0.9 m	SLFNE	KCNQ2 (AD)	chr20:62059782	NM_172107:c.154delT;p.I385TfsTer4	het	♀carrier	
120 F	1.8 m	West syndrome	ADSL (AR)	chr22:40754948	NM_000026:c.563G>A;p.R188H	comp het	♀carrier	S-Adenosyl-l-methionine trial proposed but patient died just prior to implementation
				chr22:40760969	NM_000026:c.1277G>A;p.R426H		♂carrier	
144 F	3 days	Unclassified	FGF12[a] (AD)	chr3:192053223	NM_021032:c.341G>A;p.R114H	het	De novo	Remains seizure free with normal development on carbamazepine
165 M	21.6 m	Dravet	SCN1A (AD)	chr2:166915162	NM_001165963:c.301C>T;p.R101W	het	De novo	Avoiding sodium channel blockers and started Valproic acid early
167 F	7.7 m	Unclassified	SCN8A (AD)	chr12:52200900	NM_014191:c.5630A>G;p.N1877S	het	♂carrier	Kept on Sodium channel blockers. Seizure free and normal development
193 M	1.5 m	West syndrome	ARX (XLR)	chrX:25022869	NM_139058:c.1607G>C;p.R536T	hemi	♀carrier	
197 M	4.4 m	Dravet	SCN1A (AD)	chr2:166866246	NM_001165963:c.3985C>T;p.R1329X	het	De novo	Avoiding sodium channel blockers and started Valproic acid early
200 F	8.9 m	Dravet	SCN1A (AD)	chr2:166859067	NM_001165963:c.4198delA;p.I1400X	het	Unknown	Avoiding sodium channel blockers and started Valproic acid early
201 M	4.6 m	Dravet	SCN1A (AD)	chr2:166848872	NM_001165963:c.4913T>A;p.I1638N	het	De novo	Avoiding sodium channel blockers and started Valproic acid early
205 M	3 days	EE	SCN1A (AD)	chr2:166848429	NM_001165963:c.5356C>G;p.L1786V	het	De novo	Influenced medication choice: topiramate
206 M	4.6 m	Unclassified	PRRT2 (AD)	chr16:29824973	NM_145239:c.604_607delTCAC; p.S202HfsTer26	het	♂carrier	No change in management
222 F	52.1 m	EE	MECP2 (XLD)	chrX:153296878	NM_004992:c.401C>G;p.S134C	het	De novo	

(Continued)

TABLE 2 | Continued

ID Sex	AAO	Epilepsy type	Gene (IP)	GRCh37/hg19	Variant	Zyg	Origin	Treatment Impact
234 M	0.8 w	Unclassified	ALDH7A1 (AR)	chr5:125882034	NM_001182:c.1547A>G;p.Y516C	het	♀carrier	Treated with B6, lysine restricted diet
				chr5:125887751	NM_001182:c.1279G>C;p.E427Q	het	♂carrier	
237 F	1 m	SLFIE	SCN2A (AD)	chr2:166231307	NM_021007:c.4085A>T;p.K1362M	het	♀carrier	Discontinue medication early because of predicted benign course
RETROSPECTIVE PATIENTS								
002 M	3.2 m	Dravet-like	ATP1A2 (AR)	chr1:160100072	NM_000702:c.1642C>T;p.R548C	comp het	♀carrier	Stopped stiripentol; started flunarizine. No further episodes on flunarizin
				chr1:160109762	NM_000702:c.3022C>T;p.R1008W		♂carrier	
005 F	7 m	West syndrome	ALG13 (XLD)	chrX:110928268	NM_001099922:c.320A>G;p.N107S	het	De novo	
006 M	10.4 m	LGS	GABRB3 (AD)	chr15:27017551	NM_000814:c.238A>G;p.M80V	het	Not ♀, ♂ NA	
013 F	5 m	EE	KCNQ5[a] (AD)	chr6:73821107	NM_001160133:c.1106C>G;p.P369R	het	De novo	
014 M	0.5 w	EE	PNPT1[b] (AR)	chr2:55910954	NM_033109:c.419C>T;p.P140L	hom	♀ & ♂ carrier	
023 M	11.2 m	EE	PIGA (XLR)	chrX:15344061	NM_002641:c.823C>T;p.R275T	hemi	♀carrier	
039 M	1 day	EE	KCNQ2 (AD)	chr20:62070997	NM_172107:c.881C>T;p.A294V	het	De novo	Topiramate changed to carbamazepine: no improvement in seizure frequency 6 months later
040 F	3 days	EE	KCNQ2 (AD)	chr20:62071034	NM_172107:c.844G>T;p.D282Y	het	De novo	Phenytoin changed to carbamazepine: seizures less frequent and shorter 9 months later
043 F	3 m	West syndrome	PAFAH1B1 (AD)	chr17:2577530	NM_000430:c.849_853delCTGGG;p.W292SfsTer10	het	De novo	
044 M	2.1 m	EE	SLC1A2[b] (AD)	chr11:35336636	NM_004171:c.244G>A;p.G82R	het	De novo	
050 M	13.7 m	Unclassified	TUBB2B (AD)	chr6:3226887	NM_178012:c.74G>A;p.S25N	het	De novo	
063 M	18.5 m	Unclassified	RORA[b] (AD)	chr15:60803740	NM_134261:c.505C>T;p.Q169X	het	De novo	
065 F	3.5 m	West syndrome	SLC35A2 (AD)	chr20:62070997	NM_001282651:c.550_552delTCC;p.S184del	het	De novo	Galactose trial: 6 months later more alert and interactive; no change in seizure frequency
071 F	46.5 m	Unclassified	HNRNPH2[b] (XLD)	chrX:100667593	NM_01959:c.617G>A;p.R206Q	het	Not ♀, ♂ NA	
072 M	29.2 m	Unclassified	TCF20[b] (AD)	chr22:42611134	NM_181492:c.178C>T;p.R60X	het	De novo	
073 F	12 m	EE	YWHAG[b] (AD)	chr7:75959244	NM_012479:c.394C>T;p.R132C	het	De novo	
077 F	2.1 m	West, LGS	CDKL5 (XLD)	chrX:18622288	NM_003159:c.1245_1246delAG;p.E416VfsTer2	het	De novo	
093 F	21.4 m	EE	SMARCA2[a] (AD)	chr9:2081857	NM_139045:c.2210C>A;p.S737Y	het	De novo	
094 F	7 m	Dravet	SCN1A (AD)	chr2:166915157	NM_001165963:c.305delT;p.F102SfsTer10	het	De novo	Changed medications: added clobazam and stiripentol

(Continued)

TABLE 2 | Continued

ID Sex	AAO	Epilepsy type	Gene (IP)	GRCh37/hg19	Variant	Zyg	Origin	Treatment Impact
100 F	10.1 m	Unclassified	ATP1A3 (AD)	chr19:42471896	NM_152296:c.2839G>A;p.G947R	het	De novo	No change in management. Already on Flunarizine
106 F	3.7 m	EE	STXBP1 (AD)	chr9:130413885	NM_003165:c.41T>G;p.I14S	het	De novo	
113 F	7.5 m	West syndrome	DYNC1H1 (AD)	chr14:102469031	NM_001376:c.4700G>A;p.R1567Q	het	De novo	
123 F	11.8 m	EE	GABRA1 (AD)	chr5:161309644	NM_000806:c.604C>T;p.R214C	het	De novo	Based on functional studies, will be started on new medication (pending publication)
126 F	60 m	Unclassified	RORA[b] (AD)	chr15:60803459	NM_134261:c.785dupG; p.E263RfsTer20	Het	De novo	
130 F	1.1 m	West syndrome	DEPDC5 (AD)	chr22:32239187	NM_001242896:c.2623delT; p.Y875Tfs46	het	De novo	
132 M	28.1 m	CSWS	CNKSR2 (XLR)	chrX:21609267	NM_014927:c.1785G>A; p.W595X	hemi	♀carrier	
137 F	24 m	Unclassified	DYRK1A (AD)	chr21:38865409	NM_001396:c.1042G>A;p.G348R	het	Not ♂, ♀ NA	
138 F	18 m	EE	DCX (XLD)	chrX:110653428	NM_178151:c.199G>A;p.G67R	het	De novo	
148 F	12 m	Unclassified	DYRK1A (AD)	chr21:38865409	NM_001396:c.763C>T;p.R255X	het	De novo	
152 F	7 m	EE	NEXMIF[b] (XLD)	chrX:73962510	NM_001008537:c.1882C>T;p.R628X	het	unknown	
158 F	26.4 m	MAE/Febrile seizures plus	SCN1A (AD)	chr2:166848429	NM_001165963:c.298T>G.F100V	het	De novo	No change in management
160 F	27.5 m	EE	MECP2 (XLD)	chrX:153296777	NM_004992:c.502C>T;p.R168X	het	De novo	
168 F	41.5 m	EE	MECP2 (XLD)	chrX:153296857	NM_004992:c.422A>C;p.Y141S	het	De novo	
171 F	10 m	EE	HNRNPU (AD)	chr1:245025823	NM_031844:c.817C>T;p.Q273X	het	De novo	
208 F	10.8 m	Unclassified	PCDH19 (XLD)	chrX:99662976	NM_001184880:c.619_620delCGinsA; p.R207KfsTer5	het	De novo	Trial of steroids, but no response
213 M	1 day	Unclassified	ATP1A3 (AD)	chr19:42471896	NM_152296:c.2443G>A;p.E815K	het	De novo	Started Flunarizine with reduced hemiplegic episodes
230 F	3.5 m	West, LGS	SCN8A (AD)	chr12:52099304	NM_014191:c.1238C>A;p.A413D	het	De novo	Sodium channel blocker tried, not continued
248 F	37 m	Unclassified	SCN1A (AD)	chr2:166909410	NM_001165963:c.646A>T;p.R216X	het	De novo	Influenced choice of future treatment (stiripentol, CBD oil)

[a]Variants identified by trio WES.

[b]Variants identified by WES reanalysis.

AAO, Age At Onset; AD, autosomal dominant; AR, autosomal recessive; CSWS, Epileptic Encephalopathy with continuous spike-and-wave during sleep; D, days; EE, unspecified Epileptic Encephalopathy; FS, Febrile seizure; IP, inheritance pattern; LGS, Lennox-Gastaut syndrome; MAE, Epilepsy with myoclonic-atonic seizures; M, months; NA, not available; SE, status epilepticus; SLFNE, Self-limited familial neonatal/infantile epilepsy; XL, X-linked; Zyg, zygosity; het, heterozygous; hom, homozygous; hemi, hemizygous.

[c]c.1029+1_1029+2delGTinsAA disrupts the canonical splice donor site of exon 12 and is predicted to abolish normal splicing.

Patients highlighted in bold are those for whom a genetic diagnosis had treatment implications.

Genetic Diagnosis[a]	Mean AAO (range)	Males (%)	EE (%)	Treatment Resistant[b] (%)	GDD/ID (%)	Autism (%)	MRI Abnormal (%)
Definite or Likely (n = 59)	13.2 months (0.03–60)	39	61	80	85	25	37
No Diagnosis (n = 100)	21.9 months (0.03–60)	43	46	81	76	20	30

[a]Individuals with variants of unknown significance or a possible genetic diagnosis are excluded (8).
[b]Treatment resistant refers to failure to respond to 2 or more appropriate anti-seizure medications.
AAO, Age at onset; EE, Epileptic Encephalopathy; GDD/ID, Global Developmental Delay/Intellectual Disability.

Comparative Time to Diagnosis in First 50 Patients

The mean time to genetic diagnosis from study enrolment with genetic counseling to research validation of the variant was 38 days (20–70) for the Prospective group, 54 days (22–105) for the Retrospective group, and 47 days (20–105) overall. The mean time from epilepsy diagnosis to research validation of genetic diagnosis was 145 days (42–242) for the Prospective group and 2,882 days (429–7,686) or ∼8 years for the Retrospective group.

Cost Analysis in First 50 Patients

Point estimates and 95% confidence intervals based on bootstrapped standard errors (1,000 times with replacement) for each category of diagnostic test by cohorts were calculated (**Table 4**). All cost estimates use rates effective for the 2014–2015 fiscal year. The mean total cost related to the diagnosis of epilepsy was $4,524 (range $1,223–$7,852) for the Prospective cohort and $8,344 (range $3,319–$17,579) for the Retrospective cohort. Diagnostic imaging and electrophysiological tests comprise >60% of total epilepsy-related diagnostic costs. The mean for diagnostic imaging testing constituted $1,391 and $3,276, for Prospective and Retrospective cohorts, respectively. The mean for electrophysiological testing constituted $1,353 and $2,731, for Prospective and Retrospective cohorts, respectively. Our alternative scenario for diagnostic testing is MRI, EEG, chromosome microarray (CMA) and WES testing with Sanger sequencing validation, which amounts to $3,234 per patient (**Supplementary Table 5**). The difference in mean total cost related to the diagnosis of epilepsy for Prospective ($4,524) and Retrospective ($8,344) groups, exceeds the cost of our diagnostic alternative ($3,234). The potential average savings of targeted WES in the diagnostic workup constitute $1,290 per Prospective patient and $5,110 per Retrospective patient.

DISCUSSION

High-throughput sequencing has been proven to be a great diagnostic tool in clinical practice in a variety of genetic conditions, and if used early in the diagnostic workup can lead to a reduction of costs associated with obtaining a molecular diagnosis (22). Gene panel sequencing is often favored over WES based on diagnostic yield, higher coverage and cost-savings (1). However, direct comparisons of diagnostic yield and/or costs of gene panel testing are limited. A comparative coverage analysis restricted to disease-causing variants identified through panels demonstrated that targeted WES detects ≥98.5% of those mutations (44), and targeted WES has been recently shown to have a higher diagnostic yield compared to gene panels (45). A major advantage of WES over panels is the ability to sequence the entire coding genome. Such comprehensive assessment can facilitate re-analysis for novel genes as they are implicated. In the course of this study several new epilepsy genes were identified by other research groups and published in the medical literature. We were able to go back to the original WES data and examine those genes in patients without a diagnosis. Re-analysis of WES data identified new diagnoses in eight patients (014, 044, 063, 071, 072, 073, 126, 152) and three additional diagnosis (013, 093, 144) were identified by trio WES, overall increasing the diagnostic rate from 27 to 33%. Given its static nature, panel sequencing is unable to include such contemporary targets. Newly discovered genes cannot be added to the test without re-design and validation, ultimately reducing the cost-effectiveness of gene panel testing.

The clinical utility of targeted WES with Sanger validation (limited ≤3 variants/exome) is supported by the identification of a definite or likely diagnosis in 59/180 (33%) patients and a possible diagnosis in an additional 21/180 (12%) (**Table 1**). A higher yield was found in the Prospective group with new-onset epilepsy and supports earlier testing, though the number of patients is small. However, this may also reflect increased severity of the disease, survival and potentially some bias in case referral. The Retrospective group had already undergone extensive clinical testing that was non-diagnostic. Nevertheless, our ability to still identify a genetic diagnosis supports the technology's superior resolution, while related data on phenotypes, management and outcomes may yet inform clinical practice.

The diagnostic yield in our study is comparable to previous findings (2–11). Most variants were de-novo and the genetic causes identified were heterogeneous. However, multiple variants were identified in several genes with the most common being SCN1A, followed by KCNQ2 and MECP2 (**Table 2**). In a comparable cohort, positive results were identified by WES in 112/293 (38.2%) epilepsy patients (3). We concur that the diagnostic yield is likely affected by the characteristics of the group studied, sample size, platform used (gene panel or WES) and the timing of the study, given ongoing gene discoveries in epilepsy. In our study, patients with a genetic diagnosis were younger and more likely to have an epileptic encephalopathy

	Mean	Bootstrap Std. Err.	95% CI		Range Min	Max
COMBINED DIAGNOSTIC COST						
Retrospective	$8,344.27	$556.97	$7,252.61	$9,435.90	$3,318.50	$17,578.8
Prospective	$4,524.07	$497.57	$3,548.85	$5,499.29	$1,223.18	$7,852
LAB TESTS						
Retrospective	$1,333.5	$83.71	$1,169.42	$1,497.56	$123.30	$3,129.91
Prospective	$1,151.32	$135.14	$886.43	$1,416.19	$209.75	$1,959.19
GENETIC TESTS						
Retrospective	$1,179.55	$98.80	$985.90	$1,373.20	0	$2,279.34
Prospective	$633.187	$144.27	$350.41	$915.96	0	$1,720
DIAGNOSTIC IMAGING						
Retrospective	$3,276.10	$214.10	$2,856.47	$3,695.74	$1,460	$6,836
Prospective	$1,391.38	$150.26	$1,096.86	$1,686	$630	$2,290
ELECTROPHYSIOLOGICAL						
Retrospective	$2,731.22	$376.26	$1,993.75	$3,468.70	0	$8,460.45
Prospective	$1,353.12	$315.64	$734.4	$1,971.78	$188.01	$3,572.19

when compared to patients in which no genetic cause was found. Similar to a prior study (3), patients with an epileptic encephalopathies had a high rate of positive findings (44%).

Our results support the feasibility of targeted WES to rapidly provide clinically-confirmed genetic diagnoses in early-onset epilepsy. Time to Sanger sequencing validation from enrollment averaged 7 weeks which is similar to the 6–8 week turn-around-time quoted by most commercial testing labs. However, this estimate did not include the additional time required to obtain provincial government approval, on a case-by-case basis, to fund WES.

A timely genetic diagnosis is important when considering the potential for treatment impact and optimization of patient outcomes. Twenty-seven of the fifty nine patients with a genetic diagnosis (46%) had a disorder with specific treatment implications; for 23 patients an immediate change in medical management was made (**Table 2**). The number of genetic disorders identified to have specific treatments implications is likely to grow with ongoing advances in precision medicine.

In British Columbia, the average savings are estimated to be between $1,290 and $5,110 per patient, depending on whether they are new Prospective referrals or Retrospective. Of note, price estimates reflect academic and/or hospital costs rather than commercial costs which are 1–5X higher. The Canadian sequencing costs cited are comparable to previous reports but will decrease as even higher throughput sequencing technologies become accessible (15–18). Current healthcare cost estimates are also conservative as patients without a genetic diagnosis will undoubtedly require additional clinic visits and inpatient hospital stays, including epilepsy monitoring unit admissions related to finding the cause of their condition. Of note, a targeted WES approach did not lead to a substantial increase in referrals for incidental findings. Overall, our findings show targeted WES may provide an effective end to an otherwise invasive, time consuming and costly diagnostic odyssey, with societal and economic benefits. Our results also support WES implementation beyond early-onset epileptic encephalopathies as we have examined a larger and more diverse group of children (18).

Limitations and Strengths

Our study has several limitations including small sample size although our diagnostic yield is comparable to previous studies. Incomplete coverage of the 620 genes analyzed was partially addressed as outlined in the methods. Proband-parent trio-based WES analyses were not used primarily for financial reasons. Analysis was restricted to 620 epilepsy genes, rather than the entire exome, to identify a genetic diagnosis as quickly as possible and to minimize secondary findings. Assessing relevance of secondary findings and proving pathogenicity of variants in novel candidate epilepsy genes is costly; thus, this approach was taken to maximize patient care and minimize cost. WES data from patients with initial negative results continues to be periodically reviewed for variants in newly described epilepsy genes. In subsequent WES trio analysis, a subset of families has helped identify novel genetic etiologies (41). All rare variants were considered however the Ion Proton™ sequencing platform tends to make mis-leading variant calling errors with small in/dels and homo-polymer stretches, with an excess of between 58 and 76% false positive calls (46). We have also identified instances of negative calls. Illumina sequencing platforms have better performance but assessing exon dosage from exome data is challenging at this sequencing depth. Panel sequencing, designed for more uniform coverage and greater depth, would be advantageous if the candidate gene was included or whole genome sequencing given the ability to detect all variants albeit for greater cost at lower coverage. Limited demographic information was provided for on the majority of patients and may be helpful in assessing variant frequencies in specific ethnic groups. Unfortunately, the ability to infer ancestry information

from exome data is rather limited, in contrast to high-density SNP arrays, and not entirely reliable.

Although a significant number of genetic diagnoses had potential treatment implications, the long-term impact on clinical outcome following genetically-informed therapeutic interventions is unknown. Early diagnosis and early intervention are important, but advances in precision medicine are also required.

The methods employed for cost analysis cannot replace a prospective randomized controlled trial (RCT) and may not have accurately assessed or included all healthcare costs related to an epilepsy diagnosis. However, an RCT assessing the effect of WES testing on healthcare costs is not yet a practical consideration. Our estimates are not a perfect or a complete description of the current diagnostic work-up, as test records are scattered across different electronic health records systems and paper charts. Data collation within an accessible unified health electronic record would help identify where additional savings are possible. In this study, indirect costs, and the psychosocial impact on the child and family were not measured.

CONCLUSION/SUMMARY

Targeted WES with limited Sanger sequencing validation is a rapid and minimally invasive test with potential to save costs within the Canadian healthcare system. An early genetic diagnosis may improve a patient's clinical outcome and quality of life. Further research on larger cohorts is warranted to inform diagnosis, clinical outcome and precision medicine. Acknowledging the limitations of our study, targeted WES with Sanger sequencing validation substantially improves current practice and is recommended as the dominant diagnostic strategy in early onset epilepsy. Minimally, as high-throughput sequencing costs continue to fall, trio-based whole exome sequencing reporting (and potentially re-reporting if negative), averting the need for Sanger validation for *de novo* variants, should be implemented as a first-line test strategy in British Columbia.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of BC Children's Hospital and University of British Columbia Ethics Board with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the BC Children's Hospital and University of British Columbia Ethics Board (protocol number H14-01531).

AUTHOR CONTRIBUTIONS

MD contributed to study conception and design, data analysis and interpretation, review of patients clinically, obtaining funding, drafting the manuscript. IG contributed to genetic data acquisition, analysis and interpretation, revised the manuscript. CD contributed to data acquisition, analysis and interpretation, and revised the manuscript. MM contributed to genetic data acquisition and to data analysis. CyB contributed to clinical assessments, data acquisition and interpretation, revised the manuscript. DE performed bioinformatics analysis. SB designed REDCap database, contributed to data acquisition, analysis and interpretation, revised the manuscript. ET contributed to data acquisition and organization. LH, AD, AM, KS, EL-R, and BB contributed to clinical assessments and data acquisition/interpretation. GH and RS performed biochemical clinical assessments, contributed to data interpretation. CvK contributed to study design and variant interpretation. ES assisted preparation of execution of study. PE performed cytogenetic assessment. SA performed genetic counseling, contributed to data acquisition and literature review. MV contributed to genetic counseling, review of results and literature, and revising manuscript. TN contributed to data analysis and interpretation, performed clinical variant interpretation, revised the manuscript, negotiated funding for variant clinical validation. CoB performed economics data acquisition, analysis and interpretation; drafted economics section of manuscript. MC performed electroclinical phenotyping, revised the manuscript, obtained funding. MF contributed to study conception and design, data analysis and interpretation, obtained funding, and revised the manuscript.

ACKNOWLEDGMENTS

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2019.00434/full#supplementary-material

REFERENCES

1. Dunn P, Albury CL, Maksemous N, Benton MC, Sutherland HG, Smith RA, et al. Next generation sequencing methods for diagnosis of epilepsy syndromes. *Front Genet.* (2018) 9:20. doi: 10.3389/fgene.2018.00020

2. Lemke JR, Riesch E, Scheurenbrand T, Schubach M, Wilhelm C, Steiner I, et al. Targeted next generation sequencing as a diagnostic tool in epileptic disorders. *Epilepsia.* (2012) 53:1387–98. doi: 10.1111/j.1528-1167.2012.03516.x

3. Helbig KL, Farwell Hagman KD, Shinde DN, Mroske C, Powis Z, Li S, et al. Diagnostic exome sequencing provides a molecular diagnosis for a significant proportion of patients with epilepsy. *Genet Med.* (2016) 18:898–905. doi: 10.1038/gim.2015.186

4. Allen NM, Conroy J, Shahwan A, Lynch B, Correa RG, Pena SDJ, et al. Unexplained early onset epileptic encephalopathy: exome screening and phenotype expansion. *Epilepsia.* (2016) 57:e12–17. doi: 10.1111/epi.13250

5. Della Mina E, Ciccone R, Brustia F, Bayindir B, Limongelli I, Vetro A, et al. Improving molecular diagnosis in epilepsy by a dedicated high-throughput sequencing platform. *Eur J Hum Genet.* (2015) 23:354–62. doi: 10.1038/ejhg.2014.92

6. Mercimek-Mahmutoglu S, Patel J, Cordeiro D, Hewson S, Callen D, Donner EJ, et al. Diagnostic yield of genetic testing in epileptic encephalopathy in childhood. *Epilepsia.* (2015) 56:707–16. doi: 10.1111/epi.12954

7. de Kovel CGF, Brilstra EH, van Kempen MJA, van't Slot R, Nijman IJ, Afawi Z, et al. Targeted sequencing of 351 candidate genes for epileptic encephalopathy in a large cohort of patients. *Mol Genet Genomic Med.* (2016) 4:568–80. doi: 10.1002/mgg3.235

8. Perucca P, Scheffer IE, Harvey AS, James PA, Lunke S, Thorne N, et al. Real-world utility of whole exome sequencing with targeted gene analysis for focal epilepsy. *Epilepsy Res.* (2017) 131:1–8. doi: 10.1016/j.eplepsyres.2017.02.001

9. Peng J, Pang N, Wang Y, Wang X-L, Chen J, Xiong J, et al. Next-generation sequencing improves treatment efficacy and reduces hospitalization in children with drug-resistant epilepsy. *CNS Neurosci Ther.* (2018) 25:14–20. doi: 10.1111/cns.12869

10. Yang L, Kong Y, Dong X, Hu L, Lin Y, Chen X, et al. Clinical and genetic spectrum of a large cohort of children with epilepsy in China. *Genet Med.* (2018) 21:564–71. doi: 10.1038/s41436-018-0091-8

11. Parrini E, Marini C, Mei D, Galuppi A, Cellini E, Pucatti D, et al. Diagnostic targeted resequencing in 349 patients with drug-resistant pediatric epilepsies identifies causative mutations in 30 different genes. *Hum Mutat.* (2017) 38:216–25. doi: 10.1002/humu.23149

12. Evers C, Staufner C, Granzow M, Paramasivam N, Hinderhofer K, Kaufmann L, et al. Impact of clinical exomes in neurodevelopmental and neurometabolic disorders. *Mol Genet Metab.* (2017) 121:297–307. doi: 10.1016/J.YMGME.2017.06.014

13. Chérot E, Keren B, Dubourg C, Carré W, Fradin M, Lavillaureix A, et al. Using medical exome sequencing to identify the causes of neurodevelopmental disorders: experience of 2 clinical units and 216 patients. *Clin Genet.* (2018) 93:567–76. doi: 10.1111/cge.13102

14. Thevenon J, Duffourd Y, Masurel-Paulet A, Lefebvre M, Feillet F, El Chehadeh-Djebbar S, et al. Diagnostic odyssey in severe neurodevelopmental disorders: toward clinical whole-exome sequencing as a first-line diagnostic test. *Clin Genet.* (2016) 89:700–7. doi: 10.1111/cge.12732

15. Soden SE, Saunders CJ, Willig LK, Farrow EG, Smith LD, Petrikin JE, et al. Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. *Sci Transl Med.* (2014) 6:265ra168. doi: 10.1126/scitranslmed.3010076

16. Valencia CA, Husami A, Holle J, Johnson JA, Qian Y, Mathur A, et al. Clinical impact and cost-effectiveness of whole exome sequencing as a diagnostic tool: a pediatric center's experience. *Front Pediatr.* (2015) 3:67. doi: 10.3389/fped.2015.00067

17. Stark Z, Schofield D, Alam K, Wilson W, Mupfeki N, Macciocca I, et al. Prospective comparison of the cost-effectiveness of clinical whole-exome sequencing with that of usual care overwhelmingly supports early use and reimbursement. *Genet Med.* (2017) 19:867–74. doi: 10.1038/gim.2016.221

18. Joshi C, Kolbe DL, Mansilla MA, Mason SO, Smith RJH, Campbell CA. Reducing the cost of the diagnostic odyssey in early onset epileptic encephalopathies. *Biomed Res Int.* (2016) 2016:1–8. doi: 10.1155/2016/6421039

19. Payne K, Gavan SP, Wright SJ, Thompson AJ. Cost-effectiveness analyses of genetic and genomic diagnostic tests. *Nat Rev Genet.* (2018) 19:235–46. doi: 10.1038/nrg.2017.108

20. Vrijenhoek T, Middelburg EM, Monroe GR, van Gassen KLI, Geenen JW, Hövels AM, et al. Whole-exome sequencing in intellectual disability; cost before and after a diagnosis. *Eur J Hum Genet.* (2018) 26:1566–71. doi: 10.1038/s41431-018-0203-6

21. Dragojlovic N, Elliott AM, Adam S, van Karnebeek C, Lehman A, Mwenifumbo JC, et al. The cost and diagnostic yield of exome sequencing for children with suspected genetic disorders: a benchmarking study. *Genet Med.* (2018) 20:1013–21. doi: 10.1038/gim.2017.226

22. Howell KB, Eggers S, Dalziel K, Riseley J, Mandelstam S, Myers CT, et al. A population-based cost-effectiveness study of early genetic testing in severe epilepsies of infancy. *Epilepsia.* (2018) 59:1177–87. doi: 10.1111/epi.14087

23. Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, et al. ILAE classification of the epilepsies: position paper of the ILAE Commission for Classification and Terminology. *Epilepsia.* (2017) 58:512–21. doi: 10.1111/epi.13709

24. Fisher RS, Cross JH, French JA, Higurashi N, Hirsch E, Jansen FE, et al. Operational classification of seizure types by the International League Against Epilepsy: position paper of the ILAE Commission for Classification and Terminology. *Epilepsia.* (2017) 58:522–30. doi: 10.1111/epi.13670

25. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)-A metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform.* (2009) 42:377–81. doi: 10.1016/j.jbi.2008.08.010

26. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.* (2010) 38:e164. doi: 10.1093/nar/gkq603

27. Pollard KS, Hubisz MJ, Rosenbloom KR, Siepel A. Detection of nonneutral substitution rates on mammalian phylogenies. *Genome Res.* (2010) 20:110–21. doi: 10.1101/gr.097857.109

28. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc.* (2009) 4:1073–81. doi: 10.1038/nprot.2009.86

29. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. *Nat Methods.* (2010) 7:248–9. doi: 10.1038/nmeth0410-248

30. Chun S, Fay JC. Identification of deleterious mutations within three human genomes. *Genome Res.* (2009) 19:1553–61. doi: 10.1101/gr.092619.109

31. Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods.* (2010) 7:575–6. doi: 10.1038/nmeth0810-575

32. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet.* (2014) 46:310–15. doi: 10.1038/ng.2892

33. Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipiralla S, et al. ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Res.* (2016) 44:D862–8. doi: 10.1093/nar/gkv1222

34. Steele JC, Guella I, Szu-Tu C, Lin MK, Thompson C, Evans DM, et al. Defining neurodegeneration on Guam by targeted genomic sequencing. *Ann Neurol.* (2015) 77:458–68. doi: 10.1002/ana.24346

35. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* (2015) 17:405–23. doi: 10.1038/gim.2015.30

36. Kalia SS, Adelman K, Bale SJ, Chung WK, Eng C, Evans JP, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet Med.* (2017) 19:249–55. doi: 10.1038/gim.2016.190

37. Quilichini PP, Chiron C, Ben-Ari Y, Gozlan H. Stiripentol, a putative antiepileptic drug, enhances the duration of opening of GABAA-receptor channels. *Epilepsia.* (2006) 47:704–16. doi: 10.1111/j.1528-1167.2006.00497.x

38. Stewart JD, Horvath R, Baruffini E, Ferrero I, Bulst S, Watkins PB, et al. Polymerase γ gene POLG determines the risk of sodium valproate-induced liver toxicity. *Hepatology.* (2010) 52:1791–6. doi: 10.1002/hep.23891

39. Klepper J, Scheffer H, Leiendecker B, Gertsen E, Binder S, Leferink M, et al. Seizure control and acceptance of the ketogenic diet in GLUT1 deficiency syndrome: a 2- to 5-year follow-up of 15 children enrolled prospectively. *Neuropediatrics*. (2005) 36:302–8. doi: 10.1055/s-2005-872843

40. Wilbur C, Buerki SE, Guella I, Toyota EB, Evans DM, McKenzie MB, et al. An infant with epilepsy and recurrent hemiplegia due to compound heterozygous variants in ATP1A2. *Pediatr Neurol*. (2017) 75:87–90. doi: 10.1016/j.pediatrneurol.2017.06.003

41. Lehman A, Thouta S, Mancini GMS, Naidu S, van Slegtenhorst M, McWalter K, et al. Loss-of-function and gain-of-function mutations in KCNQ5 cause intellectual disability or epileptic encephalopathy. *Am J Hum Genet*. (2017) 101:65–74. doi: 10.1016/j.ajhg.2017.05.016

42. Guella I, McKenzie MB, Evans DM, Buerki SE, Toyota EB, Van Allen MI, et al. *De novo* mutations in YWHAG cause early-onset epilepsy. *Am J Hum Genet*. (2017) 101:300–10. doi: 10.1016/j.ajhg.2017.07.004

43. Guella I, Huh L, McKenzie MB, Toyota EB, Bebin EM, Thompson ML, et al. *De novo FGF12* mutation in 2 patients with neonatal-onset epilepsy. *Neurol Genet*. (2016) 2:e120. doi: 10.1212/NXG.0000000000000120

44. LaDuca H, Farwell KD, Vuong H, Lu H-M, Mu W, Shahmirzadi L, et al. Exome sequencing covers >98% of mutations identified on targeted next generation sequencing panels. *PLoS ONE*. (2017) 12:e0170843. doi: 10.1371/journal.pone.0170843

45. Dillon OJ, Lunke S, Stark Z, Yeung A, Thorne N, Gaff C, et al. Exome sequencing has higher diagnostic yield compared to simulated disease-specific panels in children with suspected monogenic disorders. *Eur J Hum Genet*. (2018) 26:644–51. doi: 10.1038/s41431-018-0099-1

46. Seo H, Park Y, Min BJ, Seo ME, Kim JH. Evaluation of exome variants using the Ion Proton Platform to sequence error-prone regions. *PloS ONE*. (2017) 12:e0181304. doi: 10.1371/journal.pone.0181304

APPENDIX

List of Relevant Tests:
   Laboratory Services/Tests:
- Bloodspot acylcarnitines
- Plasma amino acids
- Plasma total homocysteine
- Serum ceruloplasmin
- Serum copper
- Ammonia Serum
- Lactate Serum/plasma
- Lactate whole blood
- Acylcarnitine Serum
- Homocysteine total from Plasma
- Plasma amino-acids
- Serum copper
- Serum coeruloplasmin
- Plasma very long chain fatty acids
- Plasma cholesterol
- Urine creatine metabolites
- Urine glycosaminoglycans
- Urine oligosaccharides
- Urine organic acids
- Urine purines and pyrimidines
- Amino acids-urine
- Urine creatine metabolites
- Urine mucopolysaccharides
- CSF Protein
- CSF Protein
- CSF Glucose
- CSF Cell
- CSF Lactate
- CSF Amino-Acids
- CSF Neurotransmitters
- Plasma Vit B12
   Genetic Tests:
- Chromosome microarray
- Fluorescence *in situ* Hybridization
- Single Gene testing
- Gene Panel/HTS testing
- Mitochondrial DNA analysis
   Diagnostic Imaging Tests:
- MRI
- CT
- PET
- PET/CT
- Ultrasound **Electrophysiological Tests:**
- EEG
- EMG
   Biopsies:
- Skin biopsy
- Muscle biopsy