A comparison of genomic diagnostics in adults and children with epilepsy and comorbid intellectual disability

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Abstract

Next generation sequencing provides an important opportunity for improved diagnosis in epilepsy. To date, the majority of diagnostic genetic testing is conducted in the paediatric arena, while the utility of such testing is less well understood in adults with epilepsy. We conducted whole exome sequencing (WES) and copy number variant analyses in an Irish cohort of 101 people with epilepsy and co-morbid intellectual disability to compare the diagnostic yield of genomic testing between adult and paediatric patients. Variant interpretation followed American College of Medical Genetics and Genomics (ACMG) guidelines. We demonstrate that WES, in combination with array-comparative genomic hybridisation, provides a diagnostic rate of 27% in unrelated adult epilepsy patients and 42% in unrelated paediatric patients. We observe a 2.7% rate of ACMG-defined incidental findings. Our findings indicate that WES has similar utility in both adult and paediatric cohorts and is appropriate for diagnostic testing in both epilepsy patient groups.

Introduction

Epilepsy is a common neurological disorder affecting around 50–60 million people worldwide [1]. An estimated 70–80% of patients with epilepsy are thought to have an

underlying genetic cause or predisposition, a proportion of which are monogenic which can be detected by whole exome sequencing (WES) or array-comparative genomic hybridisation (array-CGH) [2] and in this context, next-generation sequencing (NGS) offers great potential for genetic diagnostics in the clinic. To date, reports from large-scale WES projects in epilepsy have focused mainly on cohorts with severe epilepsies of infancy and childhood, particularly the epileptic encephalopathies [3–5]. These studies have reported diagnostic, monogenic causes in circa 27% of cases, identifiable via exome sequencing [5–9]. In

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contrast, less is known about the diagnostic yield of WES in adult epilepsy populations, and it is unknown if adult patients with epileptic encephalopathy who survive into adulthood have a different genetic aetiology to a paediatric patient cohort. Furthermore, it can be difficult to diagnose childhood onset epilepsy syndromes in adult patients due to the continuing development of the patient's disease phenotype.

There have been only limited efforts to establish the diagnostic yield of WES in adults with epilepsy and co-morbid learning disability specifically. Previous studies, in mixed-age populations with multiple seizure types, have reported a diagnostic yield of 33–38% [9–11]. Thevenon et al. used WES to study a cohort of 43 patients with severe epilepsy (epileptic encephalopathy) and/or non-syndromic intellectual disability, ranging in age (at time of testing) from <5 to 55 years [10]. The authors reported an overall diagnostic yield of 32.5% [10]. Schouwenaars-Snoeijs et al. reported a diagnostic yield of 28.3% in adult patients with epilepsy and intellectual disability [9]. Helbig et al. performed WES in people with seizures and intellectual disability and/or developmental delay, referred for diagnostic WES at a commercial laboratory [11]. Of the recruited 314 patients, 38 were adults. An overall diagnostic rate of 38.2% (112/293) was reported in the sequenced patients with seizures [11]. A majority of the studies mentioned above did not implement American College of Medical Genetics and Genomics (ACMG) guidelines [12] when estimating the diagnostic single nucleotide variant (SNV) yield from NGS in their cohorts [5, 7, 8, 10], which likely will have resulted in higher yields than if applying the ACMG criteria.

Similar to SNVs, the diagnostic yield of copy number variants (CNVs) is better understood in paediatric epilepsy, relative to adult patients with epilepsy [13–17]. Olson and colleagues for example, conducted microarray testing for CNVs in paediatric patients with seizures and/or epilepsy, reporting a diagnostic rate of 5% (40/323) using stringent variant classification guidelines [13, 18]. Analysing a cohort of adults with paediatric-onset epilepsy and intellectual disability, Borlot et al. reported a diagnostic rate of 16.1% (23/143) when applying ACMG guidelines [14]. Striano et al. used microarray-based analyses to study 279 adult patients with unexplained epilepsy (29% with ID), reporting a diagnostic rate of 9.3% [19].

Here, we describe the ACMG-guided diagnostic yield of genomic testing (WES and array-CGH), and associated spectrum of variants, present in a predominantly adult cohort with unexplained refractory epilepsy and intellectual disability. This study was conducted to inform the clinical utility of genomic testing in adult epilepsy patients compared with a similar cohort of children tested during the same study period.

Subjects and methods

Patient cohort

Adult and paediatric patients were recruited via tertiary referral clinical centres throughout Ireland, specifically; Beaumont Hospital, Cork University Hospital, Galway University Hospital, Our Lady's Children's Hospital Crumlin, St. James' Hospital, and the Daughters of Charity (St. Vincent De Paul). Inclusion criteria for adult patients were: a diagnosis of epilepsy with co-morbid ID, no clear cause and DNA samples available from both parents. Inclusion criteria for paediatric patients were: a diagnosis of epilepsy with co-morbid ID, no clear cause, a history of epileptic encephalopathy and DNA samples available from both parents.

Previous genetic testing with a targeted gene panel or WES, was an exclusion criteria for both adult and paediatric arms of the study. However, patients were included if they had previously undergone single gene test(s) (e.g., *SCN1A* and/or *SLC2A1* etc).

Information on the patient's medical and family history (defined as a first-degree relative with epilepsy), and phenotype was collected by the primary research team, via face-to-face interview and by manual review of patients' medical charts.

Next-generation sequencing

DNA from the proband and both parents were extracted in a research setting, from peripheral blood leucocytes (Qiagen, QIAcube), saliva or buccal swabs. A second DNA sample from the proband was extracted in an accredited laboratory setting for confirmatory DNA testing by an accredited service provider, as required. Whole-exome capture was conducted in a research setting using Roche NimbleGen SeqCap EZ V3 as per the manufacturer's instructions using 500 ng of input gDNA. Sequencing was performed on an Illumina NextSeq platform. Targeted panel sequencing was conducted using a custom Roche NimbleGen SeqCap panel (compiled on the basis of literature review, this panel was established at the start of the study and remained fixed) (Table S1) as per manufacturer's instructions using 500 ng of input DNA and sequenced using an Illumina MiSeq. Array-CGH was performed on a subset of index cases and selected parental samples as required using Agilent SureTag labelling kit and 44 K Human Genome array-CGH Microarray Kit.

Bioinformatic analyses

Exome data were aligned to the GRCh37/hg19 reference genome [20] and processed using a Burrows-Wheeler

Aligner and Picard [21]. Variants were identified using the Genome Analysis ToolKit (GATK) best practices protocol and annotated using ANNOVAR. Exomes with a minimum of 85% of target bases covered at $\geq 10\times$ were included for analysis. Relatedness between proband and parents was confirmed using identity by descent testing in PLINK [22]. Bioinformatic pipelines were hosted on a dedicated Microsoft Azure server. Following analysis using the in-house pipeline, unsolved cases were analysed using a separate pipeline via SapientiaTM v1.9, a commercially available diagnostic decision support platform by Congenica Ltd (Hinxton, UK) (www.congenica.com).

Variant classification

We selected, for discussion at a multidisciplinary team (MDT) meeting, ‘qualifying variants’ from WES that satisfied the following criteria; (1) minor allele frequency (MAF) < 1% (for a recessive model) or MAF < 0.1% (for a dominant model) in gnomAD control database [23], (2) functional (exonic/splicing variant), predicted damaging by at least two of three prediction software tools (PolyPhen [24], SIFT [25] and/or MutationTaster [26]) and (3) in a gene either known to cause epilepsy or intellectual disability (as per the Online Mendelian Inheritance in Man (OMIM) website, accessed most recently in January 2019 [27]) or included in the ACMG recommended list of clinically actionable incidental findings [28]. Candidate genes from across the exome were assessed for a seizure or intellectual disability phenotype using OMIM for each case, and variants assessed were not limited to a specific gene panel. We considered ‘qualifying’ CNVs if the event was (1) large (>1 Mb), (2) spanned a gene listed in OMIM as morbid in neurological disease and (3) was not commonly seen in control populations (<3 similar sized variants in non-disease populations in the Database of Genomic Variation [29]). The genomic results from this study were uploaded to an electronic patient record via a custom designed ‘genomics module’ [30]. This module facilitated integration of clinical and genomics data, discussion of each patient’s genomics results at an MDT meeting and sharing of clinically important results with referring clinicians throughout the Irish public healthcare system [30]. The inheritance pattern of each qualifying variant was considered during discussion at MDT. Where there were multiple affected first-degree relatives, we tested the relevant variant for segregation in additional family members. Variants which did not match the expected segregation pattern for pathogenicity were considered as benign or VUS. The MDT meetings had input from a clinical geneticist (MG), geneticist/bioinformaticians (KB, GLC), adult and paediatric neurologists (ND, NA, HE, DC, CD, MK, JL, DW, SB) and an epilepsy advanced nurse practitioner (MW).

Clinical interpretation and variant validation

Qualifying variants were classified in terms of pathogenicity according to ACMG guidelines [12, 18, 31]. Additional details on local interpretation of the ACMG guidelines are provided in the Supplementary Information (Table S2). Cascade testing was considered when requested by family members. Candidate variants were confirmed on an independent DNA sample (extracted in an accredited setting) using Sanger sequencing, array-CGH or Multiplex Ligation-dependent Probe Amplification as appropriate. Confirmation testing was conducted by an accredited service provider (CeGaT GmbH, Germany). The accredited service provider is also an independent clinical genetics interpretation of variant pathogenicity. Once the presence and pathogenicity of the candidate variant were confirmed by the accredited lab, results were returned to the patient and/or carers via the clinical geneticist. All variants discussed in this manuscript have been submitted to ClinVar (see Table 2) and requests for access to raw sequence data can be made via direct contact with the corresponding author.

Incidental findings

Adult patients were informed on the concept of incidental findings [28] during the consenting process and provided with the option whether or not to receive clinically actionable incidental findings, as previously defined by the ACMG [28, 32]. This list of clinically actionable genes can cause disorders which have preventative measures and/or treatments available. In the case of ID, the patients’ legal guardian/parent provided consent on behalf of the patient. We did not analyse for incidental findings in all paediatric patients, as per requirements of local ethics committees.

Mosaicism detection

Deep sequencing (coverage > 100 $\times$) of selected patients and their parents was considered in cases of suspected mosaicism (multiple affected siblings with apparent de novo inheritance) using a targeted Roche SeqCap EZ custom sequencing panel (Table S1).

Results

Patient cohort

A cohort of 101 adult and paediatric patients (representing 96 families) with epilepsy and co-morbid ID and their parents underwent WES, of which 80 patients also had array-CGH testing. Of the sequenced patients, 74/101 (73%) were adult (age ≥ 18 years) and 27/101 (27%) were

Table 1 Patient characteristics in adult and paediatric groups.

	Adult patients	Paediatric patients	Total patients
Number of patients	74	27	101
Age at seizure onset			
0–10 years	0	17	17
11–20 years	13	10	23
21–30 years	40	0	40
31–40 years	14	0	14
41–55 years	7	0	7
Gender (% Male)	65% (48/74)	57% (16/28)	64% (64/101)
Average number of current AEDs	4	3	3
Average number of prior AEDs	5	2	4
Family history of epilepsy	16% (12/74)	11% (3/27)	15% (15/101)
Array-CGH completed	82% (61/74)	70% (19/27)	79% (80/101)

The average age of onset presented here is a summary statistic calculated for patients with a known age at onset.

paediatric (age < 18 years). All patients had recurrent seizures. Five affected sibling pairs were recruited (four adult, one paediatric pair). Patient characteristics are provided in Table 1.

Next generation sequencing results

A mean coverage of 44× was obtained from WES (minimum 11.31× and maximum 112.6×). Index samples ($n = 16$) with coverage of <85% at 10× were re-sequenced using a custom Roche NimbleGen targeted epilepsy sequencing panel to ensure that relevant genes were covered. After identification of qualifying variants, discussion at the MDT meeting and confirmation via the accredited laboratory, 30 ACMG-classified pathogenic/likely pathogenic variants (in disease-causing genotype state) were detected in 33/101 (33%) patients and 30/96 (31%) of families. Three variants were identified in multiple, related patients (Table 2). Diagnostic variants included SNVs in 23 established epilepsy genes as well as two CNVs in established epilepsy loci.¹ Of the 28 SNVs we identified as diagnostic, 23/28 (82%) were novel variants and absent from the literature and/or relevant public databases (See Table 2). In total, 82% (23/28) of unique SNVs were de novo, one was a result of parental mosaicism, two were recessive, one was X-linked, and one was inherited from a partially affected parent. Of the SNVs, 8/28 (28.6%) were stop-gain, 3/28 (10.7%) were frameshifts and 17/28 (60.7%) were non-synonymous (See Table 2). A diagnostic rate of 30% (22/74) was obtained in adult patients and 41% (11/27) in paediatric patients. In unrelated individuals (excluding siblings), a diagnostic rate of 27% (19/70) was obtained in adult patients and 42% (11/26) in paediatric patients. We detected three diagnostic SNVs in *CHD2*; all in patients with seizure onset between 3 and 4 years of age, mild

¹ One of these CNVs (patient 84) awaits confirmation and segregation testing from accredited laboratory.

ID at the time of seizure onset and with features of autistic spectrum disorder. Two potentially disease-causing, ACMG ‘likely pathogenic’/‘pathogenic’ deletions were identified from array-CGH (See Table 2). Deep sequencing (coverage > 100×) of selected patients ($n = 3$ probands) and their parents was conducted in cases of suspected mosaicism. In one family, confirmation of mosaicism provided additional evidence of pathogenicity.

Previous evidence has suggested a correlation between paternal age >35 and epilepsy in offspring [33, 34]. In this study, the average paternal age was 33 years and the average maternal age was 30 at the time of birth. The average paternal age in trios with an identified pathogenic de novo variant was 33 years, and not significantly different to average paternal age in trios without a pathogenic de novo variant (32 years), (p value from two-tailed student’s t test: 0.57). The percentage of patients with a molecular diagnosis and age of seizure onset ≤3 years was 43% (10/23) in the adult cohort and 70% (7/10) in the paediatric cohort. This difference was not significant (Fisher’s Exact test p value: 0.197). All variants identified are shown in Table 2. Patient phenotype information is listed in Table 3. A total of 26 of the diagnostic variants were identified from the in-house GATK based bioinformatics pipeline, two by array-CGH and two using Sapientia 1.9.

Parental mosaicism

Three cases from two families were tested for parental mosaicism, and mosaicism was confirmed in one family. A shared variant in *MBD5* was detected in two affected full siblings. The variant was not identified in either parent from WES data or Sanger sequencing. Deep sequencing using our gene panel identified mosaicism in the mother, explaining the segregation pattern. *MBD5* is associated with autosomal dominant developmental delay with ID, autism, and multiple seizure types [35]. A third sibling with ID without seizures (a different phenotype from the proband) also carried the variant, identified through segregation testing. The pedigree chart is shown in Fig. 1.

Incidental findings

Four potential incidental findings were identified across *KCNH2*, *KCNQ1*, *MYH11* and *VHL* from WES. Although only two of these satisfied the ACMG guidelines (Table 2) (yield of ACMG-classified incidental findings: 2.7% (2/74)), we proceeded with confirmatory testing in all four at the accredited provider to get a second opinion, since these were all previously reported as disease-causing (ClinVar database [36]). Two of these four variants were deemed pathogenic by the accredited service provider. Following consultation with our clinical geneticist, it was

Table 2 Variants identified and associated ACMG pathogenicity classification.

ID	Age Group	Sex	Gene	Genomic coordinates (hg19)	Reference sequence	cDNA position	Variant	Inheritance	Variant Type	Previously Reported	MAF	ACMG Classification	ACMG Evidence	Detection Method	Accredited Laboratory Classification	ClinVar Accession
1	Adult	F	<i>DYNC1H1</i>	chr14:102446800	NM_001376.5	c.874 C > T	p.(R292W)	De novo	SNV	No	0	Likely pathogenic (II)	PS2, PM2, PF2, PF3	In-house	Pathogenic	SCV001027104
5	Adult	F	<i>ALDH5A1</i>	chr6:24505099 & chr6:24528285	NC_000006.11	c.[612 G > A]; [1273 C > T]	p.(W204*) p.(R412*)	Comp Het	Stopgain / Stopgain	Yes	0.0002/ 0.0001	Pathogenic (Ic) / Pathogenic (Ic)	PVS1, PM3, PM3, PP5	In-house	Known pathogenic	SCV001160778 and SCV001160779
9	Adult	M	<i>CHD2</i>	chr15:93563256	NC_000015.9	c.4921 C > T	p.(Q1641*)	De novo	Stopgain	Yes	0	Pathogenic (Ia)	PVS1, PS2, PM2, PF2, PF5	In-house	Pathogenic	SCV001160780
25	Adult	F	<i>DCX</i>	chrX:110653416	NC_000023.10	c.211 G > A	p.(A221V)	De novo	SNV	No	0	Pathogenic (IIIb)	PS2, PM2, PM5, PF2, PF3	In-house	Probably pathogenic	SCV001160781
30	Adult	M	<i>CHD2</i>	chr15:93534746	NC_000015.9	c.3454 C > T	p.(R1152W)	De novo	SNV	No	0	Likely pathogenic (II)	PS2, PM2, PF2, PF3	In-house	Unknown significance	SCV001160782
34	Adult	M	<i>PURA</i>	chr5:139494545	NC_000005.9	c.779 C > G	p.(P260R)	De novo	SNV	No	0	Likely pathogenic (II)	PS2, PM2, PF2, PF3	In-house	Probably pathogenic	SCV001160782
37 & 39	Adult	M	<i>MBD5</i>	chr2:149226238	NC_000002.11	c.726delC	p.(D242fs)	Mother	FS Deletion	No	0	Pathogenic (Ic)	PVS1, PM2	In-house	Pathogenic	SCV001160783
42	Pead	M	<i>SCN1A</i>	chr2:166915102	NC_000002.11	c.361 G > C	p.(A121P)	De novo	SNV	No	0	Likely pathogenic (II)	PS2, PM2, PF2, PF3	In-house	Probably pathogenic	SCV001160784
43	Pead	F	<i>STXBPI</i>	chr9:130435456	NC_000009.11	c.1030_1030del	p.(Y344fs)	De novo	FS Deletion	No	0	Pathogenic (Ia)	PVS1, PM2, PM2, PF3	In-house	Probably pathogenic	SCV001160785
50	Adult	F	<i>GABRA1</i>	chr5:161309645	NC_000005.9	c.641 G > A	p.(R214H)	De novo	SNV	Yes	0	Likely pathogenic (II)	PS2, PM2, PF2, PF5	In-house	Pathogenic	SCV001160787
52	Adult	F	<i>SMC1A</i>	chrX:53421723	NC_000023.10	c.2882 A > G	p.(Y961C)	De novo	SNV	No	0	Likely pathogenic (II)	PS2, PM2, PF2, PF3	In-house	Probably pathogenic	SCV001160788
55	Adult	M	<i>TSCI</i>	chr9:135778027	NC_000009.11	c.2353 C > T	p.(R786*)	De novo	Stopgain	Yes	0	Pathogenic (Ia)	PVS1, PS2, PM2, PF5	In-house	Pathogenic	VCV000048943.3
57 & 75	Adult	M	<i>FGFR3</i>	chr4:1803571	NC_000004.11	c.749 C > G	p.(P250R)	Father	SNV	Yes	4.23E-06	Likely pathogenic (III)	PS3, PF2, PF3	In-house	Pathogenic	VCV000016340.6
58	Pead	M	<i>STXBPI</i>	chr9:130438189	NC_000009.11	c.1217 G > A	p.(R406H)	De novo	SNV	No	0	Likely pathogenic (I)	PS2, PM2, PF2, PF5	Sapientia	Pathogenic	SCV001160791
59	Pead	F	CNV (<i>ATP2A1</i> , <i>TUFM</i>)	chr16:28837450-29042118	NC_000016.9		16p11.2 (205Kb)	Unknown	Deletion	No	Unknown	Pathogenic	No clear criteria	array-CGH	Pathogenic	SCV001160792
61	Adult	M	<i>NONO</i>	chrX:70518575	NC_000023.10	c.1190_1191del	p.(N397fs)	Mother	SNV	No	0	Likely pathogenic (I)	PVS1, PM2	In-house	Probably pathogenic	SCV001160793
68	Pead	M	<i>ALG13</i>	chrX:110928268	NC_000023.10	c.320 A > G	p.(N107S)	De novo	SNV	No	0	Pathogenic (II)	PS1, PS2, PM2, PF3	In-house	Likely pathogenic	SCV001160794
71	Pead	F	<i>CHD2</i>	chr15:93486194	NC_000015.9	c.948 C > A	p.(Y316*)	De novo	Stopgain	No	0	Pathogenic (Ia)	PVS1, PS2, PM2	In-house	Pathogenic	SCV001160795
73	Pead	M	<i>NEDD4L</i>	chr18:56037688	NC_000018.9	c.1822A > G	p.(M608V)	De novo	SNV	No	1.15E-04	Likely pathogenic (II)	PS2, PM2, PF2, PF3	In-house	Pathogenic	SCV001160796
83	Adult	M	<i>PAFAH1B1</i>	chr17:2579865	NC_000017.10	c.967 T > A	p.(W323R)	De novo	SNV	No	0	Likely pathogenic (II)	PS2, PM2, PF2, PF3	In-house	Pathogenic	SCV001160797
84	Pead	M	CNV (<i>RORA</i>)	chr15:61328907-61398869	NC_000015.9		15q22.2	Unknown	Deletion	No	Unknown	Pathogenic	No clear criteria	array-CGH	Awaiting confirmation	SCV001160798
88	Pead	M	<i>PPP3CA</i>	chr4:101953424	NC_000004.11	c.1339 G > T	p.(A447S)	De novo	SNV	No	0	Likely pathogenic (II)	PS2, PM2, PF2, PF3	In-house	Pathogenic	SCV001160799
89	Pead	F	<i>GRIN2A</i>	chr16:9934843	NC_000016.9	c.1447 G > A	p.(G483R)	De novo	Stopgain	No	0	Pathogenic (Ia)	PVS1, PS2, PM2	In-house	Pathogenic	SCV001160800
96	Adult	F	<i>SCN1A</i>	chr2:166859242	NC_000002.11	c.4024 G > T	p.(G1342*)	De novo	Stopgain	No	0	Pathogenic (Ia)	PVS1, PS2, PM2, PF5	In-house	Pathogenic	SCV001160801
97 & 98	Adult	F	<i>ANKRD11</i>	chr16:89341364	NC_000016.9	c.7571 A > G	p.(E2524G)	De novo	SNV	No	0	Pathogenic (IIIb)	PS2, PM1, PM2, PF2, PF3	Sapientia	Pathogenic	SCV001160802

Table 2 (continued)

ID	Age Group	Sex	Gene	Genomic coordinates (hg19)	Reference sequence	cDNA position	Variant	Inheritance	Variant Type	Previously Reported	MAF	ACMG Classification	ACMG Evidence	Detection Method	Accredited Laboratory Classification	ClinVar Accession
100	Adult	M	<i>KPTN</i>	chr19:47984017	NC_000019.9	c.598_599insTA	p.(S200fs)	Biparental (Recessive)	FS Insertion	No	0.0001	Pathogenic (lc)	PVS1, PM3, PP3	in-house	Pathogenic	SCV001160803
101	Adult	M	<i>CACNA1A</i>	chr19:13418964	NC_000019.9	c.1883C>T	p.(A628V)	De novo	SNV	No	0	Likely pathogenic (II)	PS2, PM2, PP2, PP3	in-house	Pathogenic	SCV001160804
108	Pead	F	<i>SYNGAP1</i>	chr6:33409451	NC_000006.11	c.2209 C>T	p.(Q737*)	De novo	Stopgain	No	0	Pathogenic (lc)	PVS1, PM2, PP2	in-house	Pathogenic	SCV001160805
109	Adult	F	<i>SCN1A</i>	chr2:166850722	NC_000002.11	c.4753 C>T	p.(R1585C)	De novo	SNV	No	0.0001	Likely pathogenic (III)	PS2, PP2, PP3	in-house	Pathogenic	SCV001160806
111	Adult	M	<i>PTEV</i>	chr10:89720852	NC_000010.10	c.1522 C>T	p.(R508*)	De novo	Stopgain	No	0	Pathogenic (la)	PVS1, PS2, PM2	in-house	Pathogenic	SCV001160807
Potential incidental findings:																
6	Adult	M	<i>KCNH2</i>	chr7:150647388	NC_000007.13	c.2266 A>G	p.(M756V)	Mother	SNV	Yes	0	Likely Pathogenic (II)	PS3, PM2, PP2, PP3, PP5	in-house	Unknown Significance	SCV001160808
65	Adult	F	<i>KCNQ1</i>	chr11:2790111	NC_000011.9	c.1171 C>T	p.(R391*)	De novo	Stopgain	Yes	0.0001	Pathogenic (la)	PVS1, PS2, PP5	in-house	Pathogenic	SCV001160809
96	Adult	F	<i>MYH11</i>	chr16:15814100	NC_000016.9	c.4882 A>C	p.(K1628Q)	Father	SNV	Yes	8.94E-05	VUS	PP2, PP3, PP5	in-house	Unknown Significance	SCV001160810
108	Pead	F	<i>VHL</i>	chr3:10188233	NC_000003.11	c.376 G>A	p.(D126N)	Mother	SNV	Yes	0.0001	VUS	PP3, PP5	in-house	Unknown Significance	SCV001160811
Less secure (not ACMG confirmed), noteworthy variants:																
29	Adult	F	<i>LRP2</i>	chr2:170055328 & chr2:170063464	NC_000002.11	c.[8546 G>C]; [6766 G>T]	p.(G2849A); p.(D2256Y)	Comp Het	SNV	No	0 / 0.0002	VUS / VUS	PM2, PP3 & PM2, PP3	in-house	Unknown Significance	SCV001160812 and SCV001160813
36	Adult	M	<i>OPHN1</i>	chrX:67339108	NC_000023.10	c.1343 C>T	p.(S448F)	Mother	SNV	No	0	VUS	PM2, PM3, PP3	in-house	Unknown Significance	SCV001160814

FS *deletion* frameshift deletion, SNV single nucleotide variant, *Comp Het* compound heterozygote, *VUS* variant of unknown significance, *inhouse* in-house bioinformatics pipeline (GATK based), *Sapientia* Sapientia v1.9 pipeline.

Table 3 Patient phenotypes.

ID	Sex	Gene	Age	Epilepsy phenotype	ID	Family Hx epilepsy	Seizure onset	Seizure types	Cognitive and behavioural features	Associated features	EEG	EEG abnormalities	MRI
1	F	<i>DYNC1H1</i>	Adult	Focal epilepsy	Y	N	13–18 months	GAS, GTCS	ID, BI	Did not achieve usual milestones, no regression noted, awkward gait	Abnormal	Multifocal epileptiform	Abnormal (frontal cortical dysplasia)
5	F	<i>ALDH5A1</i>	Adult	Generalised epilepsy	Y	Y	6 years	GTCS	Moderate ID, Mood disorder, BI	Hypothyroidism, chronic constipation	Abnormal	Focal slowing	NA
9	M	<i>CHD2</i>	Adult	Generalised epilepsy	Y	N	Unknown	GTCS, GTAS	ID and ASD	Slow to meet milestones, speech delay	Abnormal	Generalised epileptiform	NA
25	F	<i>DCX</i>	Adult	Generalised epilepsy	Y	N	<6 months	GTCS, CPS	Severe ID, BI, Self-injurious behaviour	Non-verbal, regression	Abnormal	Generalised epileptiform	Abnormal (bilateral pachygyria)
30	M	<i>CHD2</i>	Adult	Focal epilepsy	Y	N	4 years	GTCS, GTS	ID and ASD	Met early milestones, lack of skills progression age 4, started losing acquired skills age 6	NA	NA	NA
34	M	<i>PURA</i>	Adult	Generalised epilepsy	Y	N	16 years	GTCS	Moderate-Severe ID	Hypotonia, missed all milestones	Abnormal	Both generalised and focal epileptiform	Normal
37	M	<i>MBD5</i>	Adult	Febrile and drop seizures	Y	Y	9 months	FS, TCS, DS	Mild ID and ASD	Milestones slightly delayed	NA	NA	NA
39	M	<i>MBD5</i>	Adult	Febrile and drop seizures	Y	Y	Unknown	GTCS	Moderate ID	Non-verbal, poor social skills	Normal	Normal	Normal
42	M	<i>SCN1A</i>	Paed	Generalised epilepsy	Y	N	<6 months	CPS, FS, GTAS	Mild ID, Global development delay	Grade 2 ureteric reflux (significant family history of renal disease)	Normal and Abnormal	Normal	Normal
43	F	<i>STXBP1</i>	Paed	Generalised epilepsy	Y	N	<6 months	GTCS, GTS	Severe ID	ACTH induced cardiomyopathy and myopathy, Kyphosis thoracic spine, Scoliosis lumbar spine, hip dysplasia, myopic astigmatism	Abnormal	Epileptic encephalopathy	Abnormal (atrophy)
50	F	<i>GABRA1</i>	Adult	Generalised epilepsy	Y	N	2 years	GMS, GTCS, GAAS	Severe ID, ASD, BI	Delayed Milestones	Normal	Normal	Normal
52	F	<i>SMC1A</i>	Adult	Generalised epilepsy	Y	N	Unknown	GTCS, GMS	Moderate ID	Selective mutism	NA	NA	Normal
55	M	<i>TSC1</i>	Adult	Generalised epilepsy	Y	N	6–12 months	GTCS, GTAS	Severe ID, BI, Self-injurious behaviour	Ventral Septal Defect, Chronic ear dermatitis and otitis media, Strep Viridans Endocarditis, Chronic Renal Failure, IgA Nephropathy, Hypertension, Ungal Fibromas	NA	NA	NA
57	M	<i>FGFR3</i>	Adult	Generalised epilepsy	Y	Y	<6 months	GTAS, GTCS	Severe ID and ASD	Challenging behaviour	NA	NA	NA
75	M	<i>FGFR3</i>	Adult	Single, unprovoked event	Y	Y	<6 months		Severe ID	Delayed development, head circumference on 75th centile. Coarse features	Abnormal	Multifocal epileptiform	NA
58	M	<i>STXBP1</i>	Paed	Generalised epilepsy	Y	N	<6 months	GTCS	Global Developmental Delay	Dysmorphic features, left side weakness, weak neck, metabolic workup normal	Abnormal	Focal epileptiform	Normal (reduction in white matter noted)
59	F	<i>CNV (ATP2A1, TUFM)</i>	Paed	Generalised epilepsy	Y	N	Unknown	GAS, CPS, GMS, GTCS	ID, ASD, Pica	Non-verbal, over-weight, regression	Abnormal	Both generalised and focal epileptiform	Normal
61	M	<i>NOVO</i>	Adult	Unclassified epilepsy syndrome	Y	N	Unknown	Head shaking and staring	Severe ID	Incontinent	Abnormal	Generalised slowing	NA
68	M	<i>ALG13</i>	Paed	Generalised epilepsy, West syndrome	Y	N	6–12 months	GTCS, GCS, GTAS	Severe ID	Missed all milestones, not able to indicate needs	Abnormal	Multifocal epileptiform	NA
71	F	<i>CHD2</i>	Paed	Idiopathic generalised epilepsy	Y	N	3 years	GTCS, GMS	Mild-moderate ID	Regression from age 3, Myoclonic astatic phenotype	Abnormal	Both generalised and focal epileptiform	Normal
73	M	<i>NEDD4L</i>	Paed	Generalised epilepsy	Y	N	2 years	GAS, GTCS, GAAS, GTS, GMS	Global Developmental Delay	Hypotonia, poor coordination, strabismus right eye	Abnormal	Generalised epileptiform	NA
83	M	<i>PAFAH1B1</i>	Adult	Generalised epilepsy	Y	N	2 years	GTCS, CPS	Severe ID	Delayed milestones, non-verbal	Abnormal	Generalised epileptiform	Abnormal (lissencephaly)

Table 3 (continued)

ID	Sex	Gene	Age	Epilepsy phenotype	ID	Family Hx epilepsy	Seizure onset	Seizure types	Cognitive and behavioural features	Associated features	EEG	EEG abnormalities	MRI
84	M	<i>CNV (RORA)</i>	Paed	Focal epilepsy	Y	N	4 years	GTAS, CPS	Global Developmental Delay	Myopia, adaptive behavioural and cognitive intellectual disability, dysmorphic features	Abnormal	Multifocal epileptiform	Normal
88	M	<i>PPP3CA</i>	Paed	Paediatric epilepsy syndrome	Y	N	2 years	GTCS, GMS, GCS	Language delay, autistic features	Possible ataxia, met early milestones	Abnormal	Generalised slowing	Normal
89	F	<i>GRIN2A</i>	Adult	Paediatric epilepsy syndrome	Y	N	4 years	CPS	Speech and language delay, BI, normal development until 4 years and then regressed	Early puberty (10 years of age), tall stature	Abnormal	Multifocal epileptiform	Normal (small, ovoid fatty masses to the left of the midbrain)
96	F	<i>SCN1A</i>	Adult	Generalised epilepsy	Y	N	6–12 months	GTCS, GTAS	Moderate-Severe ID	History of febrile seizures	Abnormal	Generalised epileptiform	NA
97	F	<i>ANKRD11</i>	Adult	Generalised epilepsy	Y	Y	12 years	GTCS, GTAS	Mild ID, attends main-stream schooling	Slow to meet milestones	Abnormal	Generalised epileptiform	Normal
98	F	<i>ANKRD11</i>	Adult	Generalised epilepsy	Y	Y	11 years	GTCS, GAAS	Mild ID	Slow to meet milestones, had twin to twin transfusion syndrome	Abnormal	Generalised epileptiform	Normal
100	M	<i>KPTN</i>	Adult	Generalised epilepsy	Y	Y	4 years	GTCS	Moderate-Severe ID	Behavioural problems, contractures, dysphagia	Abnormal	Focal slowing	NA
101	M	<i>CACNA1A</i>	Adult	Focal/Generalised epilepsy	Y	N	6 years	GMS, GTCS, GTAS, GTS	Mild ID/ Learning difficulties	Oesophenia, behavioural problems	Abnormal	Generalised epileptiform	Normal
108	F	<i>SYNGAP1</i>	Paed	Focal/Generalised epilepsy	Y	N	6 years	GAS	Profound ID, global developmental delay, ASD	Did not meet milestones, non-verbal, microcephalic, poor circulation, hypotonic	Abnormal	Both generalised and focal epileptiform	Normal
109	F	<i>SCN1A</i>	Adult	Generalised epilepsy	Y	N	13–18 months	GTCS, GMS	Moderate ID	Early development normal, regression from age 9/10	Abnormal	Multifocal epileptiform	Undetermined (no acute intracranial abnormality)
111	M	<i>PTEN</i>	Adult	Generalised epilepsy	Y	N	9 years	GTCS, GMS, GTS	Mild ID	Macrocephaly, regression from 10 years of age, cough, variant asthma and eczema	Abnormal	Both generalised and focal epileptiform	Normal
Potential incidental findings:													
6	M	<i>KCNH2</i>	Adult	Idiopathic generalised epilepsy	Y	N	6 years	GTCS, GMS, GTCS, GTAS	ID	Abnormal development noted at age 6	Abnormal	Generalised epileptiform	Normal
65	F	<i>KCNQ1</i>	Adult	Generalised epilepsy	Y	N	3 months	GTCS, GMS	Severe ID	History of febrile seizures	NA	NA	NA
96	F	<i>MTH11</i>	Adult	Generalised epilepsy	Y	N	6–12 months	GTCS, GTAS	Moderate-Severe ID	History of febrile seizures	Abnormal	Generalised epileptiform	NA
108	F	<i>VHL</i>	Paed	Focal/Generalised epilepsy	Y	N	6 years	GAS	Profound ID, global developmental delay, ASD	Did not meet milestones, non-verbal, microcephalic, poor circulation, hypotonic	Abnormal	Both generalised and focal epileptiform	Normal
Less secure (not ACMG confirmed), noteworthy variants:													
29	F	<i>LRP2</i>	Adult	Generalised epilepsy	Y	N	14 years	GCS, GTCS	Severe ID	Microcephaly, bilateral hearing loss, abnormality of cerebellum	NA	NA	NA
36	M	<i>OPHN1</i>	Adult	Generalised epilepsy	Y	N	3 years	GTCS, GTAS	Mild ID	Heart murmur as a child, hypothyroidism, ataxic gait	NA	NA	Abnormal (microcephalic, arachnoid cyst)

GTCS generalised tonic clonic seizures, ID intellectual disability, NA not available, FHx family history, GTAS generalised tonic absence seizures, CPS complex partial seizures, BI behavioural issues, ASD autism spectrum disorder, GTS generalised tonic seizures, FS febrile seizures, TCS tonic clonic seizures, DS drop seizures, GTAS generalised tonic absence seizures, GMS generalised myoclonic seizures, GAAS generalised atypical absence seizures, GCS generalised clonic seizures, SPS simple partial seizures, GAS generalised atonic seizures.

Fig. 1 Pedigree chart for siblings 37 and 39 who carry an *MBD5* variant, inherited from an unaffected mother with a somatic variant. **a** (■) Probands: Epilepsy, history of febrile seizures, mild/moderate intellectual disability (ID), $\pm$ Asperger syndrome. (●) Sister of probands: Moderate ID, behavioural problems, no epilepsy. (◻) Epilepsy, no ID. Variant of Interest: *MBD5* p.(D242fs) $\pm$ = Heterozygote for *MBD5* p.(D242fs). ($\pm$) = Mosaic heterozygote for *MBD5* p.(D242fs). $-/-$ = Tested negative for *MBD5* p.(D242fs).

b II₅ Unaffected father of proband. II₆ Unaffected mother of probands. II₇ Developed epilepsy at 52 years. III₃ Proband (Patient 37): Epilepsy, mild intellectual disability (ID), Asperger syndrome, history of febrile seizures. Tetralogy of Fallot at birth. III₄ Unaffected twin sister. III₆ Moderate ID, complex mood disorder, challenging behaviour. III₇ Proband (Patient 39): Moderate ID, epilepsy, history of febrile seizures. III₁₀ Few seizures as teenager. No seizures now.

deemed important for clinical care to refer three patients (patients 6, 65 and 108) for appropriate clinical testing as a direct result of these incidental findings. The genes *KCNH2* and *KCNQ1* are associated with long-QT syndrome as well as mild seizure phenotypes.

Impact on patient care

Of the 33 patients provided with a molecular diagnosis, four findings may have a clear impact on epilepsy treatments, three *SCN1A* variants (two adults, one child) and one *TSC1* variant (adult). The patient with the *TSC1* variant may be suitable for treatment with everolimus, an mTOR inhibitor originally developed as a cancer drug shown to improve seizures in patients with tuberous sclerosis [37, 38]. In three patients with *SCN1A* variants, clinicians may choose to avoid sodium channel blockers commonly used to treat other forms of epilepsy as these medications can worsen seizures in patients with *SCN1A* variants [39, 40]. In other diagnosed patients there may be n-of-1 trials or small cases series of other therapies or management approaches which may be relevant to the phenotype, and the provision of support groups or links to communities to explore other therapeutic avenues and clinical research.

Discussion

Our findings reveal an overall diagnostic rate for WES and array-CGH of 31% in a combined adult and paediatric

cohort of unrelated patients with medically refractory epilepsy and co-morbid ID. Our findings are in line with those from comparable studies which report a diagnostic yield in mixed age cohorts of 33–38% [9–11]. The rate of molecular diagnosis was nominally higher in the paediatric cohort (41%) than the adult cohort (30%) but the difference was not statistically significant (Fisher's Exact test; $p = 0.209$). When restricted to unrelated probands, this difference remains non-significant (Fisher's Exact test; p value 0.12). Slight differences in inclusion criteria for adult and paediatric patients were used as there is no clear hallmark to distinguish between epileptic encephalopathy and intellectual disability plus epilepsy in adult epilepsy patients, some of whom may have had epilepsy since early childhood. The set of genes identified in adult versus paediatric patients were comparable, and the frequency of core epileptic encephalopathy genes (as defined by Heyne et al. [41]) was not significantly higher in the paediatric cohort (p value from Fisher's Exact test: 0.27). Our diagnostic yields are comparable to previous reports in both paediatric and mixed-age cohorts [3–7, 10, 11]. The implementation of dual-bioinformatics pipelines (in-house and via Sapientia v1.9) allowed the identification of additional variants, likely due to differences in variant-quality scoring threshold differences. A large proportion of the ACMG-classified 'likely pathogenic'/'pathogenic' variants were de novo (82%). This is consistent with previous studies emphasising the role of de novo variants in patients with epileptic encephalopathies [6, 42]. We encountered difficulties in the implementation of ACMG guidelines for both SNVs and CNVs [12, 18, 43].

For example, the ability to assign additional evidence of pathogenicity in cases where the patient inherited a candidate variant on the X chromosome from an unaffected mother would be a valuable addition to the current guidelines. To illustrate, in patient 36 (male) a qualifying variant on the X chromosome in *OPHN1*, inherited from the (unaffected) mother was not classified as pathogenic by ACMG guidelines. There is no category of evidence which can be attributed to this X-linked variant inherited from an unaffected mother (and therefore unable to cause disease in that parent) although this is pertinent information. The MDT team deemed this variant disease-causing on the basis of a close phenotypic match, but it did not satisfy ACMG criteria (Table 2). It is probable that some disease-causing variants were missed due to low WES coverage or insufficient array-CGH probes in key regions.

Whole exome sequencing for molecular diagnosis in adult epilepsy patients

The utility of WES for molecular diagnosis of patients with epilepsy and ID is established in paediatric cohorts, but the effectiveness of this approach in adult patients is less understood, with published work reporting on relatively small cohort sizes and mixed age patient populations. We demonstrate a molecular diagnostic rate of ~30% in adult patient groups with childhood-onset disease, and ID. This is due to a number of factors including the absence of genetic knowledge when these children were younger, and an under-appreciation and poor availability of appropriate genetic testing as these individuals grew into adulthood. The benefits of genomic testing to patients and their families are well established [44, 45]. A molecular diagnosis can provide clarification of diagnosis bringing a conclusion to the 'diagnostic odyssey', enhancing cost-effectiveness from alternative fruitless investigations [46] and inform prognosis. In some cases, alternative treatment options may become apparent following genetic diagnosis, for example Dravet syndrome patients with *SCN1A* variants should generally avoid carbamazepine related drugs, phenytoin and lamotrigine [47]. The differential effects of a potential precision medicine initiated in childhood versus adulthood are unknown. While interventions in childhood may improve prognosis and outcomes, interventions in adulthood may positively affect seizure control and have a considerable impact on quality of life. Genomics approaches, such as this one, are particularly relevant as we enter an era of personalised medicine which may allow for better seizure control with fewer side effects of therapy in both children and adults while increasing value from limited healthcare resources. Genomic testing can have valuable implications in terms of family planning for the patient's parents and siblings and remove any sense of blame within

families, including the adult cohort diagnosed in this study [48, 49]. Therefore, we suggest that all adult patients with epilepsy and ID of unclear cause should undergo diagnostic WES and array-CGH testing.

Incidental findings

The rate of incidental findings for adult patients consented to receive these results (2.7%) is slightly higher than reported by the ACMG (1%) [32], although it is the same as that reported by Ding et al. who predicted a rate of 2.7% based on mathematical modelling and ACMG guidelines [50]. Two of our incidental findings were in genes associated with long-QT syndrome (*KCNQ1* and *KCNH2*), but also known to infer risk of seizures [39, 40]. We speculate that any inflation in the rate of incidental findings in our cohort may indicate that the variants in genes associated with mild seizure phenotypes may, in part, contribute to the phenotype of interest. However, we have concluded that due to the patients' severe seizure profile and intellectual disabilities, these variants are unlikely to explain the entire epilepsy phenotype in these individuals.

Conclusion

This study illustrates how WES provides diagnostic and prognostic value for neurologists in adult, as well as paediatric settings. Although extremely helpful for the standardisation of variant interpretation, current ACMG guidelines require continuous development to incorporate growing evidence for pathogenicity. The next challenges are to educate health care professionals in the evolving advantages of genomic medicine, and to inform state and private funders of the clinical and cost benefit of the integration of diagnostic exome sequencing into healthcare systems for epilepsy patients with complex phenotypes [51].

Supplementary information is available at European Journal of Human Genetics' website.

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Compliance with ethical standards

Conflict of interest NJL, ASR and CAS are employed by Congenica Ltd.

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