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Genetic screening: Actionable information for epilepsy patients and clinicians

Screening for epilepsy-related gene variants can lead to effective, personalized treatment plans while reducing costs. UK and Danish scientists, led by Deb Pal, King's College London, evaluated a new service within the UK that searches for genetic variants in patients that cause epilepsy. The authors assessed the impact of next-generation gene panel tests, as well as the necessary resources to make such a service effective. Genetic testing was most effective in patients with seizure onset under two years old (21% diagnosed) and yield even higher in neonatal-onset epilepsy (63% diagnosed). For many patients with pathogenic variants, the diagnoses allowed for recommendations on treatment or enrolment in clinical trials. The researchers found that diagnostic delay and financial burden in neonatal epilepsy could be drastically reduced with gene panel testing. The scheme was highly rated by users and patients alike.

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3 **Incorporating epilepsy genetics into**
4 **clinical practice: a 360° evaluation**
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44 **Abstract**

45 We evaluated a new epilepsy genetic diagnostic and counseling service covering a UK population of 3.5
46 million. We calculated diagnostic yield, estimated clinical impact, and surveyed referring clinicians and
47 families. We costed alternative investigational pathways for neonatal onset epilepsy. Patients with epilepsy of
48 unknown aetiology onset < 2years; treatment resistant epilepsy; or familial epilepsy were referred for
49 counseling and testing. We developed NGS panels, performing clinical interpretation with a multidisciplinary
50 team. We held an educational workshop for paediatricians and nurses. We sent questionnaires to referring
51 paediatricians and families. We analysed investigation costs for 16 neonatal epilepsy patients. Of 96 patients, a
52 genetic diagnosis was made in 34% of patients with seizure onset < 2 years, and 4% > 2 years, with turnaround
53 time of 21 days. Pathogenic variants were seen in *SCN8A*, *SCN2A*, *SCN1A*, *KCNQ2*, *HNRNPU*, *GRIN2A*, *SYNGAP1*,
54 *STXBP1*, *STX1B*, *CDKL5*, *CHRNA4*, *PCDH19* and *PIGT*. Clinician prediction was poor. Clinicians and families rated
55 the service highly. In neonates, the cost of investigations could be reduced from £9,362 to £2,838 by
56 performing gene panel earlier and the median diagnostic delay of 3.43 years reduced to 21 days. Panel testing
57 for epilepsy has a high yield among children with onset < 2 years, and an appreciable clinical and financial
58 impact. Parallel gene testing supersedes single gene testing in most early onset cases that do not show a clear
59 genotype-phenotype correlation. Clinical interpretation of laboratory results, and in-depth discussion of
60 implications for patients and their families, necessitate multidisciplinary input and skilled genetic counseling.

61

62 **Keywords:** Next Generation Sequencing; effectiveness; clinical utility; diagnostic yield; genetic counselling;
63 personalized medicine; health service research; economic.

64

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66

67

68 **Introduction**

69 Genetic testing and counseling for epilepsy is now being incorporated into everyday practice in many parts of
70 the industrialised world ¹. This advance has been driven by rapid discoveries in the aetiology of rare
71 monogenic epilepsies, and technological developments in next generation resequencing (NGS)². The
72 integration of NGS testing into practice is accompanied by several challenges including clinician education,
73 results interpretation, and counseling for patients and their families ³.

74 We reflect on our experience of this transformational change from the perspective of a health service
75 provider, specifically assessing: (a) the effectiveness and utility of NGS testing, (b) the necessary inputs, and (c)
76 areas where service improvements can be made to facilitate the transition to “Precision” or “Personalised
77 Medicine”. We also asked specific questions about single vs parallel gene testing pathways based on clinician
78 predictive ability; the relative diagnostic yield for different age of onset or epilepsy syndrome; what priorities
79 clinicians and families identify; the resources necessary to provide an effective service, and whether NGS can
80 save time and money ^{4,5} using the neonatal epilepsy group as an example. We address these questions in the
81 context of a review of the initial operation of a UK regional epilepsy genetics service to a population of
82 approximately 3.5 million. To our knowledge there is limited published data from other specialist epilepsy
83 genetics services that similarly reviews their own experience⁶, therefore this study aims to fill a gap in that
84 respect. However, there are several articles on the utility of genetic testing in epilepsy and published yield ^{2,7-9}.
85 Our study aims to add to the current literature and, in addition, fill in the gaps in knowledge about how to set
86 up a tailored epilepsy genetics service, what referring clinicians and patients and families think about such a
87 service, and the cost saving implications of performing genetic testing.

88

89 **Results**

90 **Demographics.** Ninety-six unrelated eligible patients (55 male) were referred to the service, either through the
91 specialist outpatient clinic (n=40) or directly for molecular investigation through their paediatrician or
92 paediatric neurologist. All were consented for gene panel analysis. As this was a new service, many patients

93 were tested years after onset or diagnosis, including one adult patient and two post-mortem. We categorized
94 them broadly into age of onset and syndrome classes (Table 1). Sixty-four percent (49/77) were classified as
95 drug-resistant¹⁰.

96

97 **Identified variants.** Seventy-four of our ninety-six patients had previous array Comparative Genomic
98 Hybridisation (aCGH) performed (77%), of which 16 (22%) had an identified benign chromosomal
99 rearrangement. The remainder had no detected rearrangement and a normal chromosomal complement.
100 Patients with pathological findings on the aCGH do not tend to make their way into our clinic. In fact, only
101 three patients out of forty-four referred for aCGH by one of the three local clinicians we work with were found
102 to have a pathogenic chromosomal rearrangement by the local laboratory (ViaPath) and were not referred on
103 to our service: one showing Angelman's syndrome; one Klinefelter's syndrome; and one showing 9q34
104 deletion. However, the match between the epilepsy phenotype and the chromosomal rearrangement is not
105 conclusive in any of these three cases so none of these can be considered completely "solved".

106

107 For NGS panel testing, 11 patients were tested on the original **CH**ildhood Epilepsy panel containing 45 genes
108 (CHE-45); 11 on the CHE-76 (Childhood Epilepsy panel containing 76 genes); 49 on CHE-85 (Childhood Epilepsy
109 panel containing 85 genes), and 23 on CHE-102 (Childhood Epilepsy panel containing 102 genes); 2 patients
110 were referred to the epilepsy genetics service with existing positive gene panel results from another provider.
111 The gene panel itself was designed by the following co-authors: RSM, DKP and HAD. The criteria for including a
112 gene on the panel were that it should have been reported more than once in patients with monogenic
113 epilepsies. The selection of genes on the panel was regularly evaluated and updated. The panel included of
114 targeted capture of all exons and at least 5 base pairs of flanking intronic sequence of the selected genes.
115 The overall target coverage of the genes on the Amplexa CHE-46 panel was 95–97%; hence, 3–5% of the
116 regions were not analyzed, and some variations may have been missed, while the average target coverage for
117 the larger three panels was 98-99.5%. The regions missed were more or less identical across the different

118 samples, i.e. regions difficult to amplify due to high GC content, repeat elements, or regions with homology in
119 other parts of the genome.

120

121 Amplexa Genetics reporting follows the ACMG guidelines. However, there is an argument that these guidelines
122 are not very suitable for conditions with variable penetrance (which many types of epilepsy have been shown
123 to have). As our knowledge and understanding of epilepsy genetics is still limited, they do also report Class II
124 (benign) variants, and this allows us to monitor them in case our understanding changes in the future. When
125 we receive a report from Amplexa, we then compare that with our understanding of the phenotype to check
126 whether this fits with the clinical picture. This often leads us to re-grade classifications of variants reported. If,
127 however, we are still uncertain, we will request assistance from experienced colleagues in the field. Parental
128 segregation may also lead to re-classification of variant class if the results fit with the phenotype or family
129 history e.g. 95% of SCN1A variants causing SMEI will be *de novo*. Parental segregation was deemed necessary
130 when a class IV variant (defined as per the ACMG 2015 Guidelines¹¹) or above was identified in the child, or a
131 Class III variant was identified and it was in a gene that seemed to match with the child's phenotype and/or
132 family history, or in genes where *de novo* variants are usually pathogenic.

133 61% of patients (n=59) had one or more variants (Single Nucleotide Variants only - SNV) reported: 31 had only
134 benign variants; 9 had variants of unknown significance (VUS), and 19 had variants judged to be of pathogenic
135 significance. The average number of any variant, not just pathogenic, increased in line with the expansion in
136 size of the gene panel (CHE-46: 1.3; CHE-102: 1.8) indicating the additional burden of clinical interpretation¹².
137 We were constrained in our ability to retest panel negative cases because the testing was done under clinical
138 auspices and therefore no patient with initial negative results were retested on a larger panel in this study.
139 This means that the stated diagnostic yields probably underestimate what could have been achieved if
140 everyone had been tested on the most up to date panel. The average turnaround time for results was 21
141 working days, less when no variants were seen (18 days) because Sanger validation was not necessary, and
142 slightly more when parental segregation and new sample collection were necessary.

143

144 The pipeline used by Amplexa Genetics to establish pathogenicity of variants does indeed resemble other
145 genetic testing NGS models¹¹⁻¹³. The panels used were designed to cover all coding exons and exon-intron
146 boundaries of the included genes, including an additional 10 bp of the introns. Sequences were aligned to
147 hg19 using the Torrent Suite (ThermoFisher) and SNPs with a read depth ≥ 20 and variant allele frequency of
148 ≥ 0.25 were called using the Strand NGS software. Rare or low frequency variants were evaluated in an
149 internally developed pipeline. Included in this evaluation were literature and database searches like Human
150 Gene Mutation Database (HGMD), Exome Aggregation Consortium (ExAC) database, the Genome Aggregation
151 Database (gnomAD). Synonymous variants and variants in autosomal dominant genes which had been
152 observed more than 3 times or in homo-/hemizygous state in the ExAC/gnomAD database were excluded in
153 severe Epileptic Encephalopathy (EE) cases. All variants were submitted to prediction tools – predictions on
154 protein level were obtained from dbNSFP Functional Predictions and Cores 3.0 database while the variants
155 were submitted to bioinformatic software tools e.g. NNSplice and ESEfinder for predictions on transcriptional
156 level. The ACMG guidelines were applied to the resulting variants¹¹.

157 Pathogenic variants are listed in Table 3: *SCN8A* (n=4) and *SCN2A* (n=3) were the two most commonly
158 implicated genes. Two pathogenic variants were observed in *SCN1A* but not in typical *SCN1A*-associated
159 Generalised Epilepsy with Febrile Seizures (GEFS) or Dravet syndrome cases. Variants of unknown significance
160 were detected in *GABRA5*, *SCN8A*, *CHRNA2*, *RYR3*, *HNRNPU*, *CACNA1A*, *SPTAN1*, *PIGA*, *KCNQ3*, *SLC2A1*, *NPRL3*
161 and *CHRNA4* (Table 4).

162

163 **Variant yield.** The yield varied according to age of seizure onset - Table 5 shows results by patient and, if a
164 patient has several different variants they are classified by their most “serious” ranked variant
165 (pathogenic>VUS>benign). The 59 patients with at least one variant (benign, VUS and pathogenic included)
166 had a total of 54 benign variants amongst them (17 patients had more than one benign variant and 6 had one
167 or more benign variants plus a VUS or pathogenic variant as well); 9 variants of unknown significance; and 20
168 pathogenic variants (one patient had two variants in two different genes). 12 of the variants were Class 4 and
169 7 were class 5, as per the ACMG guidelines¹¹. The diagnostic yield, defined as the percentage of cases “solved”

170 by NGS panel testing was highest in the neonatal onset epilepsies (63%), intermediate in the remaining first
171 two years of life (21%), and lowest when onset was later (4%). The diagnostic yield was 23% among drug
172 resistant cases. Clinicians attempted gene prediction (by informed guesses) in 33 cases, and were correct in
173 five (15%): *SCN1A*, *PCDH19*, *GRIN2A*, *CDKL5*, *SCN2A*⁹.

174

175 **Impact.** In 63% of cases with pathogenic variants, the results had an immediate implication for treatment.

176 Most involved ion channel subunit genes such as *SCN1A*, *SCN2A*, *SCN8A*, *KCNQ2*, leading to recommendations
177 about Na⁺ blocking antiepileptic drugs in 10 cases. Two cases with acetyl-choline receptor subunit variants
178 that were suspected phenotype modifiers (*CHRNA4*, *CHRN2*) were offered experimental nicotine therapy¹⁴.

179 It should be noted that the patient with the *CHRN2* VUS did not have his treatment altered because of this
180 VUS. However, as we suspected it to be a phenotype modifier, he was offered the chance to try experimental
181 nicotine therapy as an adjunctive treatment, to see if that had any impact on his seizures. One-quarter of cases
182 were entered into a registry or research study. The families with pathogenic variants were offered expert
183 genetic counseling: in six cases (31%) an additional affected relative was diagnosed.

184

185 **Workshop and Surveys.** 19 paediatricians and epilepsy nurses attended the workshop and all offered
186 feedback. 100% agreed that the workshop was excellent and they were likely to change their practice going
187 forward. We received 10 survey responses from families (25% response), and six from clinicians (40%). Both
188 the outpatient and molecular diagnostic components of the service were rated as good or excellent (100%) by
189 clinicians. Families also rated our services highly and 100% would recommend to friends and family (Table 6).

190

191 **Investigational cost.** We retrieved complete records for 16 neonatal epilepsy patients. Total investigation
192 costs ranged from £5,094 to £15,622, average £9,362, with more than 75% of the costs allocated to
193 neuroimaging and videoEEG-telemetry. In multiple linear regression, we found statistically significant and
194 independent correlation only between diagnostic delay and cost of previous genetic tests (p=0.011).

195 Prior single gene testing among this sample included Fragile-X (*FMR1*), Ataxia-Telengectasia (*ATM*), Niemann-
196 Pick C (*NPC1*, *NPC2*), Spinal muscular atrophy (*SMN1*, *SMN2*), Prader-Willi syndrome (15q11.2-q13), Myotonic
197 Dystrophy (*DMPK*), ARX, atypical Rett syndrome (*CDKL5*), and Glutaric aciduria Type 1 (*GAT1*). Because both
198 MRI and EEG can be performed for disease monitoring as well as diagnosis, we excluded these and focused on
199 the remaining laboratory analyses performed on blood, urine and cerebrospinal fluid (CSF) samples. We found
200 that two-thirds of these costs (total average per patient: £2,004) were made up of array CGH and single gene
201 tests, as well as metabolic investigations and invasive lumbar puncture. The delay between epilepsy onset and
202 diagnosis ranged from 83 days to 17 years (median 3.4 years). Consequently, we calculated that if all neonatal
203 epilepsy patients underwent NGS panel testing as part of their first line investigations, their theoretical total
204 investigational costs would have averaged £2,838, which is £6,524 less (70%) than the actual average cost.

205

206 **Discussion**

207 NGS panel testing in epilepsy is largely effective and useful, and has particular strengths for early onset
208 epilepsies. The high diagnostic yield in the neonatal (63%) and infant (21%) onset groups is unprecedented.
209 We do not think there is any one answer as to why the yield was so high, however only selecting the most
210 appropriate patients for testing and having a good panel design are of course very important factors.
211 There is a significant impact on treatment and risk counselling for the majority of genetically diagnosed cases².
212 Families put a high value on exploring the implications of the results for their child and family; and referring
213 clinicians appreciated the quality of clinical interpretation and rapid turnaround time.
214 The inputs required are substantial and complex: in our context, they were based on an existing integrated
215 tertiary and secondary level regional epilepsy service, and relied on an educated referral base to select
216 appropriate cases, an expert multidisciplinary team for interpreting variants with clinical features, and the
217 skills of a specialized genetic counselor to translate findings into tangible benefits for families.
218 There is also a potential for huge reduction in investigation burden, cost and delay, taking into account the
219 priorities of users and referrers.

220

221 **1. Utility and Effectiveness.**

222 **1.1 Diagnostic Yield and Clinical Impact.** Yields of 10-48.5% have been reported from diagnostic NGS panels
223 consisting of 36-265 target epilepsy genes ^{7,9,15-20}, with a higher diagnostic yield in children under 2 years at
224 seizure onset. We found patients with pathogenic variants in the most common epilepsy genes *SCN8A* (n=4),
225 *SCN2A* (n=3), *SCN1A* (n=2), *KCNQ2* (n=2) and *STXBP1*, *GRIN2A*, *CHRNA4* (n=1 each), accounting for 70% of all
226 presumed disease-causing variants (Table 3). In all living cases involving Na or K channel mutations,
227 recommendations or changes were made to antiepileptic medications (AEDs). 9% of cases were entered into a
228 clinical trial; 26% of cases were entered into a phenotype registry or study awaiting future trials, and families
229 were introduced to online patient groups. Additionally, one quarter of patients had another relative diagnosed
230 following their diagnosis. The rapid turnaround time of 21 working days (14 days for urgent cases) means
231 interventions could be started in sufficient time to theoretically modify disease course or prevent
232 complications, although the evidence base for such therapies is yet to be established ²¹. In addition, we found
233 presumed pathogenic variants in epilepsy genes that have not been well characterized including *HNRNPU*, and
234 the recessive *PIGT* (homozygous). 8 of the 20 pathogenic variants have previously been published ^{7,8,15,22-32} a
235 further 3 are listed in ClinVar (Table 3); while 9 are novel.

236

237 **1.2 Single vs parallel gene testing.** The philosophy of parallel testing or “gene-first”, in patients where a
238 genetic cause is suspected but there is extensive genetic heterogeneity, is vindicated by clinicians’ limited
239 ability to predict results, and by some remarkable surprises. Clinician prediction was not often attempted and
240 we suspect this is because of the extreme genetic heterogeneity, pleiotropy, reduced penetrance and variable
241 expression in infantile onset epilepsies, these factors providing the rationale for parallel gene testing ^{33,34}. The
242 cases in which prediction was attempted reflect examples where there is better known genotype-phenotype
243 correlation. There are for example, some more specific clinical features that are characteristic of one, or a
244 handful, of genes: clustering of febrile seizures (*PCDH19*); temperature sensitivity (*SCN1A*); etc. We discuss
245 two case examples involving patients with pathogenic mutation in these genes in the discussion section.

246 Still there were many surprises as evidenced by the poor prediction rate. The full phenotypic spectra of many
247 epilepsy genes are currently being reported in the literature; as part of our continuing clinician education for
248 referring clinicians, we aim to disseminate this new knowledge to ensure that patients are accurately selected
249 for genetic testing. The following three cases deserve discussion because they demonstrate the strong clinical
250 foundation necessary for genetic testing in epilepsy.

251

252 The first was a seven-year-old child with early-onset (3 years) drug-resistant absence seizures preceded by
253 multiple febrile seizures; her mother noted the absences were sensitive to high temperature. Her father had
254 drug-responsive juvenile onset absence epilepsy. aCGH showed a paternally inherited 15q13.3 deletion, which
255 explained the familial susceptibility to absence seizures, but not the daughter's early age of onset, drug
256 resistance, febrile seizures or heat sensitivity. Gene panel testing then revealed a *de novo* mutation in *SCN1A*,
257 p.Arg1648His (Table 3).

258

259 The second had an onset of Lennox-Gastaut like symptoms in the first year of life, with severe learning
260 difficulties including developmental regression of language and motor function at the age of three. He had a
261 pattern of nocturnal motor seizures clustering over several days, repeating three times per month, and was
262 drug-resistant. NGS panel results showed pathogenic variants in *HNRNPU* (de novo) and *CHRNA4* (inherited);
263 the former explaining his overall phenotype, the second explaining his clustering nocturnal motor seizures. A
264 trial of transdermal nicotine significantly reduced his nocturnal motor seizures and improved his daytime
265 communication and functioning¹⁴. Both this case and the *SCN1A* case exemplify how "second hits" can modify
266 a seizure phenotype and also act as a focus for therapeutic modulation.

267

268 The third case had severe clusters of infantile convulsions continuing for 48-72 hours and recurring every few
269 months with intercurrent febrile illness; at age 11 years he became seizure free on levetiracetam and now
270 attends college. His clinical features resembled the seizure phenotype described in Epilepsy with Mental

271 Retardation Limited to Females³⁵. NGS panel testing surprisingly revealed a mosaic heterozygous mutation in
272 *PCDH19*. There are very few reported cases in males, and the genetic mechanism remains obscure³⁶.

273

274 In the older age group (seizure onset >2 years), the diagnostic yield was relatively low (4%). One reason is that
275 far fewer genes have been discovered in later onset epilepsies, and this should prompt us towards more
276 concerted efforts in collaborative gene discovery, especially in the focal epilepsies. However, it is likely that
277 many of these later-onset epilepsies have a more complex aetiology and so even when we discover some of
278 the associated genes, their impact on disease development will probably be modest and show wide variability
279 of penetrance and expression amongst affected individuals.

280

281 Genes for autosomal dominant sleep-related hypermotor epilepsy (ADSHE), although among the first
282 discovered (*CHRNA4*, *CHRN2*, *CHRNA2*, *KCNT1*, *DEPDC5*, *CRH*, *PRIMA1*) still only explain approximately 10% of
283 cases³⁷. Unfortunately, none of our five tested patients carried a causative mutation, suggesting that genetic
284 testing is not cost-effective in differentiating nocturnal motor phenomena in adolescents. Only recently, new
285 genes for familial focal epilepsy (FFE) have been reported from the *GATOR1* pathway (*DEPDC5*, *NPRL2*, *NPRL3*)
286 and these were missing from earlier versions of the gene panel CHE-46, CHE-76, CHE-85). While we speculate
287 that some of our FFE patients might have tested positive, we note the low (0.8-12%) current yield in sporadic
288 and FFE cases³⁸.

289

290 We also noted the low yield for children with infantile or epileptic spasms. Infantile spasms are aetiologically
291 heterogeneous: tuberous sclerosis is the most common single cause, followed by hypoxic-ischaemic injury,
292 stroke and brain malformations, and 70% of cases have abnormal MR imaging³⁹. In a recent study of 44
293 unsolved Infantile Seizures (IS) cases, 7% had a *de novo* chromosomal rearrangement, and pathogenic
294 mutations were revealed by trio exome sequencing in 28% of the remainder, suggesting that the diagnostic
295 yield can be significant in fully investigated unsolved cases⁴⁰. Among our nine unsolved cases, a complete

296 imaging, cytogenetic and metabolic screen had only been completed in one, suggesting room for better
297 workup of these cases prior to NGS panel testing.

298

299 **2. Necessary inputs**

300 **2.1 Clinical interpretation.** Variant interpretation is not always straightforward, and requires close
301 cooperation between molecular geneticist, bioinformatician, neurologist and genetic counsellor. We dealt
302 with a large volume of benign (n=54) or VUS (n=11), which represents a substantial burden for clinical
303 interpretation as well as a source of uncertainty for families. VUS arise for a number of reasons e.g.
304 inadequate bioinformatic prediction, lack of functional data, missing segregation, or incomplete knowledge of
305 genotype-phenotype correlation. In this scenario, segregation information on a novel variant only contributes
306 to diagnostic certainty when there is confidence about the bioinformatic prediction and the associated
307 epilepsy phenotype. If the evidence is scant, then proving that the change is *de novo*, or segregates with
308 disease in an affected parent will, in reality, make very little difference to the patient or family until further
309 evidence establishes the VUS as likely pathogenic, or benign. Without expert interpretation, clinicians may be
310 vulnerable to pitfalls such as over-interpreting variants as mutations or vice-versa⁴¹, and wrongly assigning
311 pathogenicity to heterozygous variants in recessive conditions.

312

313 **2.2 Clinician education and health structure.** Clinicians who understand the benefits and limitations of the
314 service are able to offer it most effectively to the right patients. Our educational workshop was very useful in
315 this regard, and most referrals that we received from workshop participants were appropriate and properly
316 worked up beforehand. Without this hierarchical structure, there is the possibility of bypassing guidelines on
317 investigation and wasting resources. However, clinical education is an ongoing process and continuing
318 feedback on outcomes and beneficial impacts are probably necessary to sustain and grow referrals and
319 appropriate NGS requests.

320

321 **2.3 Genetic counseling.** Despite universal access to the internet, many families have limited understanding of
322 the principles of human genetics and require clear and relevant information, relayed in the context of their
323 own situation before they can make an informed decision about genetic testing. Genetic counseling is the
324 process of helping people understand and adapt to the medical, psychological, and familial implications of
325 genetic contributions to disease⁴². The genetic counselor is therefore ideally placed to discuss with the family:
326 facilitating adaptation to their child's condition, discussing the process and implications of genetic testing, as
327 well as promoting informed choices, for now and in the future (e.g. family planning). A large proportion of
328 genetic epilepsies are as a result of *de novo* mutations, and so cascade testing for the wider family is often not
329 necessary. However, as germline mosaicism is now thought to be more common than it was originally,⁴³ the
330 possibility of prenatal testing in any future pregnancies is always discussed.

331

332 **3. Re-engineering services for precision medicine**

333 **3.1 Clinician and Family Feedback.** Clinicians valued the new specialist service, perceiving it helpful for
334 diagnosis, management and counseling, and 50% believed it had saved additional investigations. Referrals
335 increased over the course of the study, indicating an unmet need in the population. Families also found the
336 experience of genetic counseling and testing helpful, regardless of whether their child's case was solved or
337 not. This feedback points to the need for informed and unhurried discussion around genetic testing,
338 something that cannot be currently achieved in the current constraints of a general neurology clinic.

339 **3.2 Cost saving.** Clinician perceptions of cost-saving are supported by the analysis of neonatal epilepsy data,
340 showing that investigation costs could be reduced by two-thirds by ordering an NGS panel earlier in the
341 pathway, which has been noted before^{2,4,5}. This might also reduce the median diagnostic delay from 3.43
342 years to 21 days and feasibly allow the early use of disease-modifying drugs. However, true cost-savings are
343 likely to be less than the theoretical and would need to be calculated using a prospective study design,
344 preferably with a non-NGS tested concurrent control group. Such calculations may need to be repeated as
345 technology evolves. Nevertheless, guideline revision requires consensus and commitment from multiple
346 organizational stakeholders.

347 **Limitations.** While a prospective design has many advantages in terms of selection bias, there are a couple of
348 limitations of this study. First, because our clinical pathway separates children with primary epilepsy from all
349 children with early-onset seizures, and requires a routine workup to exclude lesional and some metabolic
350 causes as well as excluding single gene testing for *SCN1A* and *SLC2A1*, the results may not be generalizable to
351 other health care contexts. Second, our diagnostic yield concealed some variability because of the evolution of
352 the gene panel over the period of study, reflecting the fast pace of gene discovery – this might have led to
353 some under-diagnosis of patients using earlier panels.

354

355 **Methods**

356 **Ethics.** a.) methods were performed in accordance with relevant regulations and guidelines and b.) methods
357 were approved by The Great Ormond Street Hospital/Institute of Child Health Research Ethics Committee
358 (reference number: 09/H0713/76).

359

360 **Population.** We collected prospective data related to genetic testing on 96 patients referred to the King's
361 Health Partners epilepsy genetics service for molecular diagnostic testing, between November 2014 and
362 September 2016. The service is provided to the southeast region of England, a population of approximately 3.5
363 million including the south-east of London. The region includes two teaching hospitals with tertiary paediatric
364 neurology departments (King's College Hospital NHS Trust and Evelina London Children's Hospital) and eleven
365 district general hospitals in which there is a general paediatrician with a special interest in epilepsy. Medical
366 services are state-run and organized through a regional clinical network with common management guidelines
367 for epilepsy⁴⁴. Patients are seen first at their district general hospital before being referred, if appropriate, for
368 a tertiary specialist opinion either at one of the two tertiary centres or in a regional specialist epilepsy clinic.
369 The epilepsy genetics service comprises two components: a specialist clinic run by a paediatric epileptologist
370 with a research interest in genetics (DKP), a genetic counselor (SO) and clinical fellow (ST, RR); and a molecular
371 genetic diagnostic service using an NGS epilepsy panel (LHGL, QH, HAD), with clinical interpretation by the
372 whole team.

373 **Pathway.** In the absence of consensus guidelines, we considered patients suitable for genetic testing with
374 either early-onset (<2 years) epilepsy, treatment resistant epilepsy of unknown cause, or familial epilepsy
375 where the genetic cause was unknown. Two of our patients sadly died during the testing process. As a rule, we
376 only considered patients with epilepsy as their primary diagnosis, rather than patients with intellectual
377 disability (ID) or autism (ASD) who had seizures as part of their phenotype. This is because our service is part of
378 the epilepsy service, whereas patients with primary ID or ASD who also have seizures do not usually use our
379 pathway, unless they have a relevant family history. Patients followed one of three pathways for genetic
380 testing: either being seen (i) in the specialist epilepsy genetic clinic, as above (n=40); (ii) by a paediatric
381 neurologist (n=7) or paediatric epileptologist (n=37) at one of the two tertiary centres; or (iii) seen by a general
382 paediatrician (n=12) with a special interest in epilepsy at a district general hospital, with referrals made in
383 discussion with their linked paediatric epileptologist. Patients were recommended to have completed routine
384 aetiological investigations as per regional guidelines (EEG, MRI, metabolic as necessary), and the clinician was
385 asked to complete a proforma summarizing the electroclinical phenotype, epilepsy syndrome, age at seizure
386 onset, drug response, results of previous investigations, and clinical prediction of candidate gene. We collected
387 aCGH data in cases where it had been performed. Children with suspected typical Dravet Syndrome (OMIM
388 607208) or Glut-1 Deficiency syndromes (OMIM 606777) undergo single gene testing and were not included
389 here; patients with brain malformations are tested on a separate gene panel and also not discussed here.
390

391 At the outpatient visit, we spent approximately one hour with each new patient. The paediatric epileptologist
392 and genetic counsellor took a detailed clinical and genetic history and performed a neurological examination
393 on the affected child. Patients were operationally categorized into broad epilepsy syndromes (Table 1)
394 because many did not fit into the International League Against Epilepsy (ILAE) classification of epilepsy
395 syndromes⁴⁵. The genetic counselor then discussed the possibility of NGS panel testing, and if the family were
396 interested, proceeded to explain: the process; benefits and limitations; potential outcomes and what they
397 might mean; discussed any issues of concern that might arise around results, obtained written informed
398 consent (using the appended consent form) prior to the start of this study, and planned for follow-up.

399 **Education.** We held a half-day educational workshop aimed at regional paediatricians and epilepsy nurses, to
400 discuss which patients were suitable for testing, which test to choose and how to obtain informed consent.
401 We designed the educational workshops along evidence-based lines for effective learning, using case-based
402 simulations in small groups⁴⁶⁻⁴⁸. After the workshop, attendees gave anonymous feedback indicating that 100%
403 of them were “likely” or “very likely” to change their practice. We circulated proposed guidelines for genetic
404 testing to the group which were agreed in consensus. Following this, in actual practice we have seen the
405 number of referrals increase and that most referrals meet our published guidelines. Furthermore, the number
406 of new referrers has increased and as we provide email feedback to every referrer, appropriateness is also
407 improving amongst new referrers. Additionally, we posted separate information for clinicians and families on
408 our website www.childhood-epilepsy.org.

409
410 **Gene Panel.** We used the Amplexa Genetics epilepsy gene panel CHE-46 (46 epilepsy genes) at the start of the
411 service⁷, which was updated to CHE-76, CHE-85 and CHE-102 during the study period in light of new gene
412 discoveries (by DKP, RM, HAD), (Table 2). To identify putative disease-causing variants, we performed targeted
413 NGS of 46-102 epilepsy genes in four successive panels (January 2014 – January 2016). The criteria for
414 including a gene on the panel were that it should have been reported more than once in patients with
415 monogenic epilepsies. The genes included on the CHE-46 panel were: *ALDH7A1, ALG13, ARHGEF9, CACNA1A,*
416 *CDKL5, CHD2, CPA6, DEPDC5, DNMT1, GABRA1, GABBR1, GABBR2, GABRB3, GABRD, GABRG2, GNAO1, GRIN1,*
417 *GRIN2A, GRIN2B, HCN1, HDAC4, HNRNPU, IQSEQ2, KCNA2, KCNQ2, KCNQ3, KCNT1, KCTD7, LGI1, MBD5,*
418 *PCDH19, PLCB1, PNPO, PRRT2, SCN1A, SCN1B, SCN2A, SCN8A, SLC25A22, SLC2A1, SLC35A3, SPTAN1, STX1B,*
419 *STXBP1, SYNGAP1, and TBC1D24; additionally for CHE-76: ADSL, ATP1A2, ATP1A3, ATRX, CHRNA2, CHRNA4,*
420 *CHRN2, GABRA5, GAMT, GATM, MECP2, MEF2C, MTOR, PIGA, PIK3AP1, PNKP, POLG, PURA, RYR3, SLC25A2,*
421 *SLC6A1, SLC6A8, SLC9A6, SMARCA2, TCF4, UBE3A; and additionally for CHE-85: CLCN2, CNKRS2, FASN, FOXG1,*
422 *HDAC4, HNRNPU, HUWE1, KCNH5, KCTD7, MBD5, PIGO, PIGT, RELN, SIK1, SLC13A5, SLC35A2, SLC35A3,*
423 *SLC6A8, SLC9A6, ZDHHC9; and for CHE-102: CACNB4, CUX2, EEF1A2, GRIN2D, KANK1, KCNB1, KCNMA1,*

424 *KIAA2022, NPRL2, NPRL3, PIK3R2, ST3GAL3, SZT2, WWOX*. aCGH, where performed, was conducted using an
425 oligonucleotide array with ~60 000 probes across the genome. Paternity testing was not performed.

426

427 **Sample Preparation.** Genomic DNA was extracted from blood using standard methods. For the CHE-45, panel
428 libraries were prepared from 15 ng of template DNA using the Ion AmpliSeq library 2.0 kit and custom primers
429 following the manufacturer's instructions (ThermoFisher Scientific)⁷. The CHE-76, CHE-85 and CHE-102 panel
430 libraries were prepared from 1000 ng template DNA, Agilent SureSelect target enrichment (Agilent
431 technologies) and KAPA library preparation kit (KAPA Biosystems) following manufacturer's instructions. The
432 library DNA was clonally amplified onto the Ion Spheres Particles (ISPs) by emulsion PCR using an Ion
433 OneTouch 2 system and the Ion PGM Template OT2 200 kit (ThermoFisher Scientific). ISPs were sequenced on
434 an Ion PGM sequencer using an Ion 314, Ion 316 or Ion 318 chip and the Ion PGM 200 Sequencing kit as per
435 the manufacturer's instructions (ThermoFisher Scientific).

436

437 **Bioinformatics.** Sequences were mapped to hg19 in the Torrent suite software (ThermoFisher Scientific) and
438 variant calling was achieved in the Strand NGS software (Avadis) with a minimum of 20-fold read depth.
439 Common SNPs with an allele frequency $\geq 2\%$ and SNPs observed in more than 2 samples for each analyzed
440 sample batch were filtered out. Genetic nonsynonymous/splice site variants were evaluated through database
441 searches: dbSNP, Exome Variant Server, the Exome Aggregation Consortium database (ExAC), the Genome
442 Aggregation Database (gnomAD) and HGMD Professional. Missense variants were also submitted to prediction
443 softwares such as SIFT and PolyPhen-2, while splice site variants were evaluated by NNSplice and Splicesite
444 finder. Variants analyzed under a dominant inheritance model that were observed more than 10 times in ExAC
445 were considered too common as monogenic causes. Potentially pathogenic variants were validated through
446 conventional Sanger sequencing, and, if possible, parents were included for segregation analysis when
447 indicated.

448

449 **Criteria for Assessing Pathogenicity of Rare Variants.** We share the brief clinical summary of the patient with
450 the laboratory to aid genotype-phenotype correlation; subsequently we interpret the gene panel report in
451 detailed clinical context at a monthly multidisciplinary meeting including epileptologists (EH, REW, KL, DKP), a
452 clinical neurophysiologist (SG) and genetic counselor (SO). We also consulted bioinformatics databases, patient
453 registries, expert colleagues and published literature. Laboratory reported variants categorized by the ACMG
454 system¹¹ were then (re-)classified by us as either benign variants, VUS, or pathogenic variants for the purposes
455 of genetic counselling. For predicted possibly damaging variants where segregation analysis could be
456 performed, we required the variant to meet one of the following criteria to constitute a likely pathogenic
457 variant: *de novo* in early-onset severe epilepsy syndromes, segregation with the disorder, inheritance from an
458 unaffected parent but previously reported in other families with the same phenotype and incomplete
459 penetrance, or adherence to a recessive X-linked or parent-of-origin mode of inheritance.

460

461 **Result feedback.** We offered either a telephone or face-to-face consultation to the family, followed up with a
462 written summary of the discussions in a letter.

463

464 **Opinion survey.** We solicited the views of all 40 families through an anonymous 16-item questionnaire
465 available as paper copy or web version (www.surveymonkey.com). The questionnaire covered three main
466 topics of quality, impact and perceived value, and was formulated with the assistance of the Head of Patient
467 Experience at one of the tertiary centres. We also sent an email link to a 10-item anonymous
468 (www.surveymonkey.com) questionnaire to all 15 clinicians who had referred patients to the epilepsy genetics
469 service (questions were adapted from a longer survey used in the evaluation of *SCN1A* testing⁶).

470

471 **Investigational cost.** We searched electronic patient records to generate a list and timing of all investigations
472 ordered in the neonatal epilepsy group; then matched these against 2017 hospital tariffs, separating them into
473 categories of neuroimaging; EEG; routine blood tests; metabolic investigations of blood, urine and CSF; tissue

474 biopsy; array CGH and karyotype; single gene tests; and NGS panel. We assessed the independent association
475 of imaging, EEG, metabolic and genetic tests with diagnostic delay in days using multiple linear regression.

476

477 **Data Availability.** All supporting data can be found as presented in this paper.

478

479 **Conclusion.** NGS-based genetic testing has high clinical utility in children with epilepsy onset before two years
480 or in drug-resistant or familial cases. The impacts are numerous and range from treatment change to risk
481 counseling, and potential recruitment to clinical trials as new experimental therapies become available. A
482 successful service requires strong engagement from secondary health care providers, an existing framework
483 for specialist referral and investigation, substantial collaboration between clinicians and scientists for variant
484 interpretation, as well as expertise in genetic counseling and flexibility in communicating with and meeting the
485 evolving needs of families. To make the best of any innovation in medicine, health care organisations need to
486 be open to change and reconfiguration of resources to benefit patients and their families.

487

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495

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497

498 **Contributions.**

499 Stephanie Oates: writer and co-editor of paper; formulated, sent out and collated data from questionnaires.

500 Dr Shan Tang: phenotyping of patients and co-editor of paper

501 Dr Richard Rosch: phenotyping of patients and gave comments on the paper

502 Rosalie Lear: economic analysis

503 Dr Elaine Hughes: phenotyping of patients and gave comments on the paper

504 Dr Ruth Williams: phenotyping of patients and gave comments on the paper

505 Dr Karine Lascelles: phenotyping of patients and gave comments on the paper

506 Line HG Larsen: DNA extraction, NGS panel testing, Sanger Sequencing, and bioinformatic analysis

507 Qin Hao: DNA extraction, NGS panel testing, Sanger Sequencing, and bioinformatic analysis

508 Dr Hans Atli Dahl: bioinformatic analysis and gave comments on the paper

509 Dr Rikke S Møller: clinical interpretation and gave comments on the paper

510 Professor Deb Pal: Guarantor, and assisted with writing and editing of the paper

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526 **References**

- 527 1 Pal, D. K., Pong, A. W. & Chung, W. K. Genetic evaluation and counseling for epilepsy. *Nat*
528 *Rev Neurol* **6**, 445-453, doi:10.1038/nrneurol.2010.92 (2010).
- 529 2 Berg, A. T. *et al.* Early-Life Epilepsies and the Emerging Role of Genetic Testing. *JAMA*
530 *Pediatr*, doi:10.1001/jamapediatrics.2017.1743 (2017).
- 531 3 Poduri, A., Sheidley, B. R., Shostak, S. & Ottman, R. Genetic testing in the epilepsies-
532 developments and dilemmas. *Nat Rev Neurol* **10**, 293-299, doi:10.1038/nrneurol.2014.60
533 (2014).
- 534 4 Joshi, C. *et al.* Reducing the Cost of the Diagnostic Odyssey in Early Onset Epileptic
535 Encephalopathies. *BioMed research international* **2016**, 6421039, doi:10.1155/2016/6421039
536 (2016).
- 537 5 Tan, T. Y. *et al.* Diagnostic Impact and Cost-effectiveness of Whole-Exome Sequencing for
538 Ambulant Children With Suspected Monogenic Conditions. *JAMA Pediatr*,
539 doi:10.1001/jamapediatrics.2017.1755 (2017).
- 540 6 Brunklaus, A. *et al.* The clinical utility of an SCN1A genetic diagnosis in infantile-onset
541 epilepsy. *Dev Med Child Neurol* **55**, 154-161, doi:10.1111/dmcn.12030 (2013).
- 542 7 Møller, R. S. *et al.* Gene Panel Testing in Epileptic Encephalopathies and Familial Epilepsies.
543 *Mol Syndromol*, doi:10.1159/000448369 (2016).
- 544 8 Butler, K. M. *et al.* De novo and inherited SCN8A epilepsy mutations detected by gene panel
545 analysis. *Epilepsy Res* **129**, 17-25, doi:10.1016/j.epilepsyres.2016.11.002 (2017).
- 546 9 Trump, N. *et al.* Improving diagnosis and broadening the phenotypes in early-onset seizure and
547 severe developmental delay disorders through gene panel analysis. *Journal of medical genetics*
548 **53**, 310-317, doi:10.1136/jmedgenet-2015-103263 (2016).
- 549 10 Kwan, P. *et al.* Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task
550 Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia* **51**, 1069-1077,
551 doi:10.1111/j.1528-1167.2009.02397.x (2010).
- 552 11 Richards, S. *et al.* Standards and guidelines for the interpretation of sequence variants: a joint
553 consensus recommendation of the American College of Medical Genetics and Genomics and
554 the Association for Molecular Pathology. *Genetics in medicine : official journal of the*
555 *American College of Medical Genetics* **17**, 405-424, doi:10.1038/gim.2015.30 (2015).
- 556 12 Eilbeck, K., Quinlan, A. & Yandell, M. Settling the score: variant prioritization and Mendelian
557 disease. *Nat Rev Genet* **18**, 599-612, doi:10.1038/nrg.2017.52 (2017).
- 558 13 Muzzey, D., Evans, E. A. & Lieber, C. Understanding the Basics of NGS: From Mechanism to
559 Variant Calling. *Curr Genet Med Rep* **3**, 158-165, doi:10.1007/s40142-015-0076-8 (2015).
- 560 14 Brodtkorb, E. & Picard, F. Tobacco habits modulate autosomal dominant nocturnal frontal lobe
561 epilepsy. *Epilepsy Behav* **9**, 515-520, doi:10.1016/j.yebeh.2006.07.008 (2006).
- 562 15 Lemke, J. R. *et al.* Targeted next generation sequencing as a diagnostic tool in epileptic
563 disorders. *Epilepsia* **53**, 1387-1398, doi:10.1111/j.1528-1167.2012.03516.x (2012).
- 564 16 Carvill, G. L. *et al.* Targeted resequencing in epileptic encephalopathies identifies de novo
565 mutations in CHD2 and SYNGAP1. *Nat Genet* **45**, 825-830, doi:10.1038/ng.2646 (2013).
- 566 17 Kodera, H. *et al.* Targeted capture and sequencing for detection of mutations causing early
567 onset epileptic encephalopathy. *Epilepsia* **54**, 1262-1269, doi:10.1111/epi.12203 (2013).
- 568 18 Wang, J., Gotway, G., Pascual, J. M. & Park, J. Y. Diagnostic yield of clinical next-generation
569 sequencing panels for epilepsy. *JAMA neurology* **71**, 650-651,
570 doi:10.1001/jamaneurol.2014.405 (2014).
- 571 19 Della Mina, E. *et al.* Improving molecular diagnosis in epilepsy by a dedicated high-throughput
572 sequencing platform. *European journal of human genetics : EJHG* **23**, 354-362,
573 doi:10.1038/ejhg.2014.92 (2015).

- 574 20 Mercimek-Mahmutoglu, S. *et al.* Diagnostic yield of genetic testing in epileptic
575 encephalopathy in childhood. *Epilepsia* **56**, 707-716, doi:10.1111/epi.12954 (2015).
- 576 21 Chapman, K. E., Specchio, N., Shinnar, S. & Holmes, G. L. Seizing control of epileptic activity
577 can improve outcome. *Epilepsia* **56**, 1482-1485, doi:10.1111/epi.13109 (2015).
- 578 22 Vaher, U. *et al.* De novo SCN8A mutation identified by whole-exome sequencing in a boy
579 with neonatal epileptic encephalopathy, multiple congenital anomalies, and movement
580 disorders. *J Child Neurol* **29**, NP202-206, doi:10.1177/0883073813511300 (2014).
- 581 23 Singh, R., Jayapal, S., Goyal, S., Jungbluth, H. & Lascelles, K. Early-onset movement disorder
582 and epileptic encephalopathy due to de novo dominant SCN8A mutation. *Seizure* **26**, 69-71,
583 doi:10.1016/j.seizure.2015.01.017 (2015).
- 584 24 Larsen, J. *et al.* The phenotypic spectrum of SCN8A encephalopathy. *Neurology* **84**, 480-489,
585 doi:10.1212/WNL.0000000000001211 (2015).
- 586 25 Anand, G. *et al.* Autosomal dominant SCN8A mutation with an unusually mild phenotype.
587 *European journal of paediatric neurology : EJPN : official journal of the European Paediatric
588 Neurology Society* **20**, 761-765, doi:10.1016/j.ejpn.2016.04.015 (2016).
- 589 26 Parrini, E. *et al.* Diagnostic Targeted Resequencing in 349 Patients with Drug-Resistant
590 Pediatric Epilepsies Identifies Causative Mutations in 30 Different Genes. *Human mutation* **38**,
591 216-225, doi:10.1002/humu.23149 (2017).
- 592 27 Wagnon, J. L. & Meisler, M. H. Recurrent and Non-Recurrent Mutations of SCN8A in
593 Epileptic Encephalopathy. *Frontiers in neurology* **6**, 104, doi:10.3389/fneur.2015.00104
594 (2015).
- 595 28 Zara, F. *et al.* Genetic testing in benign familial epilepsies of the first year of life: clinical and
596 diagnostic significance. *Epilepsia* **54**, 425-436, doi:10.1111/epi.12089 (2013).
- 597 29 Weckhuysen, S. *et al.* KCNQ2 encephalopathy: emerging phenotype of a neonatal epileptic
598 encephalopathy. *Ann Neurol* **71**, 15-25, doi:10.1002/ana.22644 (2012).
- 599 30 Depienne, C. *et al.* Genetic and phenotypic dissection of 1q43q44 microdeletion syndrome and
600 neurodevelopmental phenotypes associated with mutations in ZBTB18 and HNRNPU. *Hum
601 Genet* **136**, 463-479, doi:10.1007/s00439-017-1772-0 (2017).
- 602 31 Lemke, J. R. *et al.* Mutations in GRIN2A cause idiopathic focal epilepsy with rolandic spikes.
603 *Nat Genet* **45**, 1067-1072, doi:10.1038/ng.2728 (2013).
- 604 32 von Stulpnagel, C. *et al.* Epilepsy in patients with GRIN2A alterations: Genetics,
605 neurodevelopment, epileptic phenotype and response to anticonvulsive drugs. *European
606 journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology
607 Society* **21**, 530-541, doi:10.1016/j.ejpn.2017.01.001 (2017).
- 608 33 Grayton, H. M., Fernandes, C., Rujescu, D. & Collier, D. A. Copy number variations in
609 neurodevelopmental disorders. *Prog Neurobiol* **99**, 81-91,
610 doi:10.1016/j.pneurobio.2012.07.005 (2012).
- 611 34 Parker, L., Howlett, I. C., Rusan, Z. M. & Tanouye, M. A. Seizure and epilepsy: studies of
612 seizure disorders in *Drosophila*. *Int Rev Neurobiol* **99**, 1-21, doi:10.1016/B978-0-12-387003-
613 2.00001-X (2011).
- 614 35 Scheffer, I. E. *et al.* Epilepsy and mental retardation limited to females: an under-recognized
615 disorder. *Brain* **131**, 918-927, doi:10.1093/brain/awm338 (2008).
- 616 36 Terracciano, A. *et al.* PCDH19-related epilepsy in two mosaic male patients. *Epilepsia* **57**, e51-
617 55, doi:10.1111/epi.13295 (2016).
- 618 37 Tinuper, P. *et al.* Definition and diagnostic criteria of sleep-related hypermotor epilepsy.
619 *Neurology* **86**, 1834-1842, doi:10.1212/WNL.0000000000002666 (2016).
- 620 38 Hildebrand, M. S. *et al.* A targeted resequencing gene panel for focal epilepsy. *Neurology* **86**,
621 1605-1612, doi:10.1212/WNL.0000000000002608 (2016).

622 39 Poulat, A. L. *et al.* A proposed diagnostic approach for infantile spasms based on a spectrum of
623 variable aetiology. *European journal of paediatric neurology : EJPN : official journal of the*
624 *European Paediatric Neurology Society* **18**, 176-182, doi:10.1016/j.ejpn.2013.11.005 (2014).
625 40 Michaud, J. L. *et al.* The genetic landscape of infantile spasms. *Hum Mol Genet* **23**, 4846-4858,
626 doi:10.1093/hmg/ddu199 (2014).
627 41 McCarthy, J. J. & Mendelsohn, B. A. (2016).
628 42 National Society of Genetic Counselors' Definition Task, F. *et al.* A new definition of Genetic
629 Counseling: National Society of Genetic Counselors' Task Force report. *Journal of genetic*
630 *counseling* **15**, 77-83, doi:10.1007/s10897-005-9014-3 (2006).
631 43 GajECKa, M. Unrevealed mosaicism in the next-generation sequencing era. *Molecular genetics*
632 *and genomics : MGG* **291**, 513-530, doi:10.1007/s00438-015-1130-7 (2016).
633 44 National Institute for Health and Care Excellence. The Diagnosis and Management of the
634 epilepsies in adults and children in primary and secondary care/cg137. (National, Institute for
635 Health and Care Excellence, 2012).
636 45 Berg, A. T. *et al.* Revised terminology and concepts for organization of seizures and epilepsies:
637 report of the ILAE Commission on Classification and Terminology, 2005-2009. *Epilepsia* **51**,
638 676-685, doi:EPI2522 [pii]
639 [10.1111/j.1528-1167.2010.02522.x](https://doi.org/10.1111/j.1528-1167.2010.02522.x) (2010).
640 46 Raza, A., Coomarasamy, A. & Khan, K. S. Best evidence continuous medical education. *Arch*
641 *Gynecol Obstet* **280**, 683-687, doi:10.1007/s00404-009-1128-7 (2009).
642 47 Davis, D., Galbraith, R., American College of Chest Physicians, H. & Science Policy, C.
643 Continuing medical education effect on practice performance: effectiveness of continuing
644 medical education: American College of Chest Physicians Evidence-Based Educational
645 Guidelines. *Chest* **135**, 42S-48S, doi:10.1378/chest.08-2517 (2009).
646 48 Bluestone, J. *et al.* Effective in-service training design and delivery: evidence from an
647 integrative literature review. *Hum Resour Health* **11**, 51, doi:10.1186/1478-4491-11-51 (2013).
648 49 Schwarz, J. M., Cooper, D. N., Schuelke, M. & Seelow, D. MutationTaster2: mutation
649 prediction for the deep-sequencing age. *Nat Methods* **11**, 361-362, doi:10.1038/nmeth.2890
650 (2014).
651

Age at seizure onset	Syndrome	Number	Age at testing median years, (range)
Neonatal (0-1 mo)	NEE	14	3.75 (0.2-16.9)
	Benign neonatal	2	0.2 (0.2)
Infantile (2-24 mo)	Infantile EE	19	7.5 (0.3-22.9)
	FS/TLE spectrum	4	6.1 (1.3-18.3)
	Infantile spasms	11	6.5 (0.5-12.2)
Childhood (>2y)	NFLE/SHE	6	13.7 (5.6-17.6)
	Generalised (LGS-like)	9	15.1 (3.4-19.9)
	Early-onset absence	4	7.45 (1.4-14.7)
	Epilepsy-Aphasia spectrum	11	10.8 (7.3-17.2)
	Familial focal epilepsy	8	10.45 (4.0-14.5)
	Refractory focal epilepsy	8	9 (4.4-17.4)
Total		96	7.5 (0.2-22.9)

Table 1. Demographics of patients for gene panel testing

NEE – Neonatal Epileptic Encephalopathy (epilepsy with onset between birth and three months of age)

IEE - Infantile Epileptic Encephalopathy (epilepsy with onset between four and 12 months)

GEFS – Generalised Epilepsy with Febrile Seizures (<https://www.epilepsydiagnosis.org/syndrome/fbp-overview.html>)

TLE – Temporal Lobe Epilepsy (<https://www.epilepsydiagnosis.org/syndrome/other-familial-temporal-lobe-overview.html>)

NFLE – Nocturnal Frontal Lobe Epilepsy also known as SHE – Sleep-related Hypermotor Epilepsy ³⁷

LGS – Lennox-Gastaut syndrome (<https://www.epilepsydiagnosis.org/syndrome/lgs-overview.html>)

Ion transport	Neuro-transmitter related	Gene expression	Scaffolding and Trafficking	Intracellular Signalling	Other Functions
<i>ATP1A2</i> <i>ATP1A3</i> <i>CACNA1A</i> <i>CACNA1H</i> <i>CACNB4</i> <i>CLCN2*</i> <i>HCN1</i> <i>KCNA2</i> <i>KCNB1</i> <i>KCND2</i> <i>KCNH5</i> <i>KCNH8</i> <i>KCNMA1</i> <i>KCNQ2</i> <i>KCNQ3</i> <i>KCNQ5</i> <i>KCNT1</i> <i>KCTD7*</i> <i>LG11</i> <i>SCN1A</i> <i>SCN1B</i> <i>SCN2A</i> <i>SCN8A</i> <i>SLC12A5</i> <i>SLC6A1</i> <i>SLC9A6</i>	<i>CHRNA2</i> <i>CHRNA4</i> <i>CHRNA2</i> <i>GABBR1</i> <i>GABBR2</i> <i>GABRA1</i> <i>GABRA5</i> <i>GABRB3</i> <i>GABRD</i> <i>GABRG2</i> <i>GRIN1</i> <i>GRIN2A</i> <i>GRIN2B</i> <i>GRIN2D</i> <i>NRXN1</i> <i>PRRT2</i> <i>SLC1A2</i> <i>SLC25A22</i> <i>SYNGAP1</i>	<i>ARX</i> <i>ATRX</i> <i>CHD2</i> <i>CUX2</i> <i>EEF1A2</i> <i>FOXG1</i> <i>HDAC4</i> <i>HNRNPU</i> <i>HUWE1</i> <i>MBD5*</i> <i>MECP2</i> <i>MEF2C</i> <i>PURA</i> <i>SMARCA2</i> <i>TCF4</i> <i>ZDHHC9</i>	<i>CNKSR2</i> <i>DNM1</i> <i>IQSEC2</i> <i>KANK1</i> <i>KIAA2022</i> <i>PCDH19</i> <i>PIGA</i> <i>PIGO</i> <i>PIGT</i> <i>RELN</i> <i>SPTAN1</i> <i>STX1B</i> <i>STXBP1</i> <i>TBC1D24</i>	<i>DEPDC5</i> <i>GNAO1</i> <i>MTOR</i> <i>NPRL2</i> <i>NPRL3</i> <i>PIK3R2</i> <i>PLCB1</i> <i>RYR3*</i> <i>SIK1</i>	<i>ADSL</i> <i>ALDH7A1</i> <i>ALG13</i> <i>ARHGEF9</i> <i>CDKL5</i> <i>CPA6</i> <i>FASN</i> <i>GAMT</i> <i>GATM</i> <i>PIK3AP1</i> <i>PNKP</i> <i>PNPO</i> <i>POLG</i> <i>ST3GAL3</i> <i>SLC13A5</i> <i>SLC2A1</i> <i>SLC35A2</i> <i>SLC35A3</i> <i>SLC6A8</i> <i>SZT2</i> <i>UBE3A</i> <i>WWOX</i>
CHE-45, CHE-76 additions to CHE-45, CHE-85 additions to CHE-76, CHE-102 additions to CHE-85 *Genes removed from CHE-102					

Table 2: Gene Panels used in this study, categorized by their function

Phenotype	Gene	Variant(s) c.DNA change	Amino Acid change	Inheritance	SIFT prediction	POLYphen prediction	gnomAD prediction	Variant published
NEE	SCN8A	c.3979A>G	p.Ile1327Val	Unknown	DAM	DAM	0	^{17,18}
NEE		c.4883T>G	p.Leu1628Trp	Unknown	DAM	DAM	0	No
IEE		c.5630A>G	p.Asn1877Ser	<i>De novo</i>	DAM	DAM	0	¹⁹⁻²²
FFE		c.5615G>A	p.Arg1872Gln	Paternal (mosaic)	DAM	DAM	1/246048	²³
BFNIS	SCN2A	c.623T>A	p.Val208Glu	Paternal (aff)	DAM	DAM	0	⁸
NEE/MIMPSI		c.640T>C	p.Ser214Pro	<i>De novo</i>	DAM	DAM	0	^{14**} single case, ClinVar, 372557
NEE		c.1312G>A	p.Glu438Lys	Unknown	DAM	DAM	0	single case, ClinVar 207057
FFE	SCN1A	c.4871T>A	p.Leu1624Gln	Maternal (aff)	DAM	DAM	0	No
EO-ABS*		c.4943G>A	p.Arg1648His	<i>De novo</i>	DAM	DAM	0	No
BFNIS	KCNQ2	c.476G>A	p.Gly159Glu	Awaited	DAM	DAM	0	²⁴
NEE		c.1678C>T	p.Arg560Trp	<i>De novo</i>	DAM	DAM	0	²⁵
NEE, LD	HNRNP1	c.1681delC	p.Gln561SerfsTer45	<i>De novo</i>	DAM	DAM	0	^{26**}
	CHRNA4 [^]	c.1454G>A	p.Arg485Gln	Maternal	DAM	DAM	40/17759	3 cases ClinVar 197690 (2 VUS)
ABPE-ESES	GRIN2A	c.2179G>A	p.Ala727Thr	Paternal	DAM	DAM	0	²⁷
IEE	SYNGAP1	c.1766T>A	p.Ile589Asn	<i>De novo</i>	DAM	DAM	0	No
NEE	STXBPI	c.1282C>T	p.Gln428Ter	<i>De novo</i>	DAM	DAM	0	No
Gen sz, DD, ASD	STX1B	c.563dupA	p.Gln189AlafsTer5	Unknown	DAM	DAM	0	No
IS, DD, VI	CDKL5	c.2177_2168delCTTCCA TGainsAATGTCAAC	p.Ser726Ter	Unknown	DAM	DAM	0	No
FS clusters*	PCDH19	c.344_345insT (exon 1)	p.Val117GlyfsTer109	<i>De novo</i> ; mosaic male	DAM	DAM	0	No
NEE	PIGT	c.709G>C (homozygous)	p.Glu237Gln	Recessive	BEN	DAM	16/24350 2 (N/A)	No

Table 3. Pathogenic, or likely pathogenic variants in 18 cases. NEE – Neonatal epileptic encephalopathy; IEE – infantile epileptic encephalopathy; BFNIS – benign familial neonatal-infantile seizures; MIMPSI – malignant migrating partial seizures of infancy; EO-ABS – early onset absence seizures; FFE – Familial Focal Epilepsy; heat-sens – heat sensitive seizures; LD – learning disability; ABPE-ESES – atypical benign partial epilepsy with electrical status in slow-wave sleep; DD – developmental delay; ASD – autism spectrum disorder; VI – cortical visual impairment;

DAM – damaging; BEN – benign; TOL – tolerated;

*see text for details

[^] CHRNA4 likely to be a modifier in this patient

** Same patient, published previously

Phenotype	Gene	Variant(s) c.DNA change	Amino Acid change	Inheritance	SIFT outcome	POLYphen outcome	gnomAD outcome	Comments
FFE LGS	GABRA5 SCN8A	c.86+1G>A c.659G>A	- p.Arg220His	Paternal Unknown	- DAM	- DAM	3/27718 2 0	Broken splice site predicted
NEE, LD, ASD*	CHRNA2	c.1378C>G	p.Arg460Gly	Maternal	TOL	DAM	108/277 170	3 cases ClinVar 191352 (2 VUS; 1 Likely benign)
ABS, regression	RYR3	c.573A>G	p.Ile191Met	Maternal	BEN	DAM	0	No
IEE	HNRNP1	c.2197_2199delAGG	p.Arg733del	Unknown	DAM	DAM	0	No
NEE	CACNA1A	c.1854G>T	p.Leu618Phe	Unknown	DAM	DAM	0	No
NEE	SPTAN1 PIGA	c.6178G>A c.1A>G	p.Glu2060Lys p.Met1?	Unknown Unknown	BEN BEN	BEN	0 0	
IEE	RYR3	c.4471G>A	p.Asp1419Asn	Unknown – not maternal	BEN	DAM	0	Disease causing in Mutation Taster http://www.mutationtaster.org/cgi-bin/MutationTaster/MutationTaster69.cgi?new_base=A&transcript_stable_id=ENST00000389232&sequence_typer=CDS
>2 FOC	SLC2A1	c.586C>G	p.Pro196Ala	Maternal	BEN	DAM	0	
FOC	NPRL3 CHRNA4	c.103C>G c.77-8C>T	p.Pro35Ala	Unknown Unknown	BEN BEN	BEN BEN	1/30884 0	Disease causing in Mutation Taster ⁴⁹

Table 4. Variants of unknown significance. FFE- familial focal epilepsy; LGS – Lennox-Gastaut syndrome; FOC – focal epilepsy; DAM – damaging; BEN – benign.

* This variant was considered a potential modifier, due to the severe presentation

Age at seizure onset	Patients with				Total	Diagnostic Yield
	no variants	only benign variants	VUS	pathogenic variants		
0-1m	1	3	2	10	16	63%
2-24m	14	10	3	7	34	21%
IEE	5	7	3	4	19	21%
FS/TLE	2	0	0	2	4	50%
IS	7	3	0	1	11	*9%
>2y	22	18	4	2	46	4%
NFLE/SHE	4	2	0	0	6	0%
GGE	4	3	2	0	9	0%
EOABS	0	4	0	0	4	0%
ESES	5	5	0	1	11	9%
FFE	4	2	1	1	8	13%
DRE-FOC	5	2	1	0	8	0%
Grand Total	37	31	9	19	96	20%

Table 5. Variant yield by age of onset and epilepsy syndrome

* one further case was subsequently solved through whole genome research investigation

Clinicians' Opinions	
Do you think that genetic testing..	Yes
..helped you to confirm or refine an existing or suspected clinical diagnosis?	83%
..has allowed a diagnosis to be made earlier than with clinical and EEG data alone?	83%
..saved your patient from additional investigations?	50%
..results altered your treatment and/or management approach?	67%
..results prevented the prescription of drugs that could have worsened the epilepsy?	17%
..was helpful in providing an explanation of the underlying disease for the family?	83%
Families views	
Question	Strongly/ Agree
How helpful was genetic testing in giving you a cause for your child's Epilepsy?	70%
Did the healthcare professionals give you enough opportunity to ask questions?	100%
Did the healthcare professionals explain things in a way you could understand?	100%
How helpful did you find it to attend the specialist outpatient clinic?	100%
How likely are you to recommend our service to friends or family who need similar care?	100%

Table 6. Referring clinicians' opinions and families' views of the epilepsy genetics service