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## Patients with KCNH1 -related intellectual disability without distinctive features of Zimmermann-Laband/Temple-Baraitser syndrome

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1 **Patients with *KCNH1*-related intellectual disability without distinctive fea-**  
2 **tures of Zimmermann-Laband/Temple-Baraitser syndrome**

3  
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43 **WORD COUNT:** text 1997 words; abstract 200 words

44 **ABSTRACT**

45 *De novo* missense variants in *KCNH1* encoding Kv10.1 are responsible for two  
46 clinically recognizable phenotypes: Temple-Baraitser Syndrome (TBS) and  
47 Zimmermann-Laband Syndrome (ZLS). The clinical overlap between these two  
48 syndromes suggests that they belong to a spectrum of *KCNH1*-related en-  
49 cephalopathies. Affected patients have severe intellectual disability (ID) with or  
50 without epilepsy, hypertrichosis and distinctive features such as gingival hyper-  
51 plasia and nail hypo/aplasia (present in 20/23 reported cases).

52 We report a series of seven patients with ID and *de novo* pathogenic *KCNH1*  
53 variants identified by whole-exome sequencing or an epilepsy gene panel in  
54 whom the diagnosis of TBS/ZLS had not been first considered. Four of these  
55 variants, p.(Thr294Met), p.(Ala492Asp), p.(Thr493Asn) and p.(Gly496Arg), were  
56 located in the transmembrane domains S3 and S6 of Kv10.1 and one,  
57 p.(Arg693Gln), in its C-terminal cyclic nucleotide binding homology domain  
58 (CNBHD). Clinical reappraisal by the referring clinical geneticists confirmed the  
59 absence of the distinctive gingival and nail features of TBS/ZLS.

60 Our study expands the phenotypical spectrum of *KCNH1*-related encephalopa-  
61 thies to individuals with an attenuated extra-neurological phenotype preventing  
62 a clinical diagnosis of TBS or ZLS. This subtype may be related to recurrent  
63 substitutions of the Gly496, suggesting a genotype-phenotype correlation and,  
64 possibly, to variants in the CNBHD domain.

65 **KEYWORDS**

66 *KCNH1*, intellectual disability, epilepsy, Temple-Baraitser syndrome, Zimmer-  
67 mann-Laband syndrome

## 68 INTRODUCTION

69 Gain-of-function missense variants in *KCNH1* (MIM\*603305) encoding the volt-  
70 age gated potassium channel Kv10.1 are associated with syndromic neurode-  
71 velopmental disorders with overlapping phenotypes referred to as Temple-  
72 Baraitser syndrome (TBS;MIM#611816) and Zimmermann-Laband syndrome  
73 (ZLS;MIM#135500).[1-7] The main features of these syndromes (Table S1) are  
74 severe intellectual disability (ID) with or without early-onset epilepsy, congenital  
75 hypertrichosis, anomalies of the limbs and nails, gingival hyperplasia and mild  
76 craniofacial dysmorphic features.[1-3, 7-13] Gingival hyperplasia is a classical  
77 feature of the ZLS phenotype, present in about 50% of the published cases of  
78 *KCNH1*-related encephalopathy.[11-14] In patients with TBS, thumbs and hal-  
79 luses are usually long, proximally implanted and broad with hypoplastic nails  
80 and an increased radiolucency with missing ossification points in the terminal  
81 phalanx.[5, 15] The other fingers and toes may show hypoplastic distal phalan-  
82 ges and nails.[10] Hypo/aplasia of nails has been reported in more than 90% of  
83 published cases with *KCNH1* variants.[4, 5, 7, 15, 16] These findings in a con-  
84 text of severe encephalopathy are highly suggestive of TBS/ZLS.

85 We report on seven novel patients with *KCNH1*-related ID and lacking the dis-  
86 tinctive features of TBS/ZLS.

87

## 88 METHODS

89 We used clinical networks (EPIGENE, EuroEPINOMICS-RES, GeneMatcher) to  
90 collect a multicentric cohort of patients with *KCNH1*-related encephalopathy,  
91 evaluated by clinical geneticists for the etiological diagnosis of ID. Referring

92 physicians retrospectively completed a standardized table with molecular and  
93 clinical data. We obtained written consents for genetic testing and publication of  
94 data for all patients. Retrospectively, craniofacial features, limbs and nails were  
95 assessed using patients' photographs.

96 Patients P1, P4 and P5 were studied on epilepsy gene panels used in different  
97 French University hospitals, including 90 to 116 genes involved in epilepsy,  
98 among 2450 individuals tested in different French university hospitals. Patients  
99 P2, P3, P6 and their parents were studied by solo or trio-WES (supplementary  
100 methods). The variant found in patient P2 was identified by performing WES in  
101 a research project on genetic ataxias. The variants in patients P3 and P6 were  
102 found by WES in the diagnosis workup of ID out of 1294 analyzed patients. Pa-  
103 tient P7 was recruited through GeneMatcher and the *KCNH1* variant was found  
104 by WES (1/850 tested patients).

105 Sanger sequencing was used for confirming variants.

106 Sequence variations were numbered using the adenine of the ATG initiation  
107 codon as the first nucleotide (*KCNH1* GenBank accession NM\_172362).

108 We performed an exhaustive review of the literature using the PubMed data-  
109 base to compile clinical data on individuals with pathogenic variants in *KCNH1*  
110 (Table S1) using the following terms: « *KCNH1*, TBS, ZLS, Temple-Baraitser,  
111 Zimmermann-Laband ». Because genetic heterogeneity has been  
112 documented,[13, 17] we excluded patients without mutation to focus on the fre-  
113 quency of clinical signs in proven *KCNH1*-related encephalopathy.

## CLINICAL DATA OF OUR 7 PATIENTS WITH *KCNH1*-RELATED ENCEPHALOPATHY

	P2	P3	P4	P5	P6	P7	TOTAL
	F	M	F	F	F	F	n=7
Age at diagnosis	15 y	7.9 y	9 y	1.75 y	3.5 y	28 y	1.75 y
Ethnicity	European	Mauritian	European	European	European	European	
Genotype	c.210977496G>T	c.210977485C>T	c.210948724C>T	c.210977485C>T	c.210977485C>T	c.210977493G>T	c.211192276G>A
Genotype	c.475C>A	c.1486G>A	c.2078G>A	c.1486G>A	c.1486G>A	c.1478C>A	c.881C>T
Ala492Asp	p.(Gly496Arg)	p.(Arg693Gln)	p.(Gly496Arg)	p.(Gly496Arg)	p.(Thr493Asn)	p.(Thr294Met)	
PS2 PM1 PM2 PP2 PP3	5 (PS2 PM1 PM2 PP2 PP3 PP5)	5 (PS2 PM1 PM2 PP2 PP3)	5 (PS2 PM1 PM2 PP2 PP3 PP5)	5 (PS2 PM1 PM2 PP2 PP3 PP5)	5 (PS2 PM1 PM2 PP2 PP3)	5 (PS2 PM1 PM2 PP2 PP3)	
IQ	29.9	26	29.9	29.9	26.7	25.7	
IQ <i>de novo</i>	<i>de novo</i>	<i>de novo</i>	likely <i>de novo</i> *	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>	
Severe ID	moderate ID	severe ID	mild/moderate ID	mild DD	moderate/severe ID	severe ID	mild -
Motor milestones	10 m	10 m	12 m	9.5 m	26 m	NA	9.5 m
Language milestones	21 m	35 m	20 m	18 m	not achieved	18 m	18 m - 4.5 y
Communication	sentences	few words	sentences	canonical babbling	no words	no words	sentent words
Seizures	yes	no	yes	yes	no	no	4/7
Seizure type	12 m	NA	2 y	3 m	NA	NA	day 1
Seizure description	tonic, tonic-clonic; 2 status epilepticus	NA	focal clonic, generalized tonic-clonic (total 5 seizures), myoclonic	generalized tonic-clonic, focal clonic, myoclonic	NA	NA	
Seizure frequency	monthly/weekly	NA	1-3/month	8/day	NA	NA	
EEG background	normal	NA	focus of central spikes increased during sleep (close to CSWS)	few spikes in central regions, bursts of generalized polyspikes with myoclonic jerks	slow background activity	normal	
Seizure response	responsive	NA	responsive	responsive with relapse	NA	NA	
Medications	levetiracetam, lamotrigine, carbamazepine and clobazam	NA	carbamazepine	levetiracetam	NA	NA	
Motor milestones	normal	normal	normal	normal	normal	normal	normal
Visual	hyperopia	none	none	none	none	none	
Motor and peripheral hypotonia	truncal hypotonia, hyperreflexia, mild ataxia	hyperreflexia	NA	normal	truncal hypotonia	facial hypotonia, hyperreflexia	

## 1 PATIENTS AND RESULTS

2 We collected clinical and molecular data of seven unrelated patients with path-  
3 ogenic *KCNH1* variants (Table 1).

4 Three individuals shared the same previously reported *de novo* (or assumed as  
5 *de novo*) heterozygous missense variant c.1486G>A, p.(Gly496Arg). The others  
6 carried novel *de novo* heterozygous missense variants [c.881C>T  
7 p.(Thr294Met), c.1475C>A p.(Ala492Asp), c.2078G>A p.(Arg693Gln) and  
8 c.1478C>A p.(Thr493Asn)] fulfilling the criteria of pathogenicity (Figure 1A, Ta-  
9 ble1).

10 All seven patients, aged 21 months to 28 years, presented with mild/moderate  
11 to severe developmental delay (DD) or ID (Table1). The age of independent  
12 walking was mildly delayed (18 to 35 months) for five patients, while the others  
13 had not achieved walking at last examination (3.5 and 4.5 years). Two out of the  
14 five patients older than 3 years were able to make sentences, one spoke a few  
15 words and the remaining three did not speak. Four patients had epilepsy start-  
16 ing in infancy, with generalized tonic-clonic (4/4), myoclonic (2/4), focal motor  
17 (2/4) and tonic seizures (1/4). One of them experienced status epilepticus. The  
18 epilepsy was pharmaco-responsive in all individuals: a monotherapy was effec-  
19 tive in three patients (2/3 levetiracetam, 1/3 carbamazepine) and one individual  
20 needed a bitherapy (carbamazepine and clobazam). Cerebral MRI was normal  
21 in all cases. P1 had a moderate sensorineural deafness.

22 At examination, the most frequent neurological sign was truncal hypotonia, brisk  
23 tendon reflexes and ataxia. The mild dysmorphic features noted in some of the  
24 patients were regarded as nonspecific by the physicians and did not guide the



25 molecular diagnosis. Referring clinicians reassessed the phenotypes of their  
26 patients after the identification of the *KCNH1* variant with special attention to the  
27 morphology of limbs and gums (Figure S1). Only hypertrichosis of the back was  
28 present in P1. Almost all patients had constipation (6/7), sometimes severe, and  
29 near half of them (3/7) had gastroesophageal reflux disease (GERD).

30

## 31 **DISCUSSION**

32 We provide a clinical description of seven individuals with *KCNH1*-related en-  
33 cephalopathy without features of TBS/ZLS found by WES or with an epilepsy  
34 gene panel. Only subtle and non-distinctive dysmorphic features were noted in  
35 some of them (Figure S1). Only one patient had mild toenails hypoplasia, but  
36 this was inherited from his mother and maternal grandfather. Three patients  
37 were reported with slightly broad halluces, two with proximal placement of  
38 thumbs, including one having long fingers.

39 Twenty-three patients with *KCNH1*-related encephalopathy have been reported  
40 to date (Table S1).[4-9, 13-16, 18, 19] Most of them had a clinical diagnosis of  
41 TBS/ZLS based on distinctive clinical features prior to molecular analysis. Two  
42 published series reported eight patients with pathogenic *KCNH1* variants found  
43 by WES[4, 16] without prior clinical diagnosis of TBS or ZLS, but all had gingival  
44 hyperplasia and/or nail anomalies in retrospect, except one who carried the  
45 Gly496Glu variant.[16] Thus, 22/23 previously reported patients with *KCNH1*-  
46 related encephalopathy had a prospective or retrospective diagnosis of  
47 TBS/ZLS with either nail hypo/aplasia or gingival hypertrophy. In comparison,  
48 the patients of the present series stand out for their attenuated extra-

49 neurological phenotype and the lack of clinical clue suggesting TBS or ZLS. Do  
50 these patients have a different developmental or epileptic outcome than  
51 TBS/ZLS patients?

52

53 All 23 previously reported patients with *KCNH1*-related encephalopathy had  
54 DD/ID. When mentioned (n=16), the ID was rarely mild (n=1), mild/moderate  
55 (n=1), or moderate (n=1), and most frequently severe (n=8), severe/profound  
56 (n=2) or profound (n=3). The range of DD/ID in our patients is consistent with  
57 the literature (Table 1). Seven out of 14 previously reported patients over 18  
58 months achieved walking at a mean age of 3.3 years, while 4/6 of our series  
59 walked at a mean age of 2 years. Ten out of 12 previous patients older than 2  
60 years had not acquired spoken language. In our series, two individuals out of  
61 five spoke words or sentences. Thus, language abilities may be mildly better in  
62 the patients of our study.

63 Epilepsy was frequent in our series (n=4/7) and in the literature (n=19/22). First  
64 seizures occurred between the first day of life and the age of 2 years (mean age  
65 1 year) in our patients, and at a median age of 0.9 year (mean age 2.5 years) in  
66 the literature (n=18). Seizures were mainly motor in nature, either focal or gen-  
67 eralized, including clonic, tonic-clonic, tonic and myoclonic seizures, both in the  
68 literature and in our series. EEG revealed unilateral or bilateral spikes or spikes-  
69 waves in fronto-centro-temporal regions in three of our epileptic patients. In the  
70 literature, multifocal epileptiform anomalies, sometimes predominating in anteri-  
71 or or fronto-temporal regions[15] and foci of spikes in frontal and central areas  
72 were both reported.[16] One of our patients experienced convulsive status epi-

73 lepticus and one had an EEG close to continuous spikes and waves during  
74 sleep. Comparatively, status epilepticus was reported in five patients of the lit-  
75 erature. We conclude that the severity of the neurodevelopmental involvement  
76 in patients of our series bears comparison with patients of the literature, with  
77 regard to both the level of DD/ID and the epilepsy.

78 Nail anomalies and gingival hypertrophy were reported in 90% and 50% of pa-  
79 tients in the literature, respectively (Table S1). The lack of nail anomalies and  
80 gingival hyperplasia in our series is noteworthy and suggests an overestimation  
81 of their frequency in *KCNH1*-encephalopathy. We cannot exclude, however, the  
82 appearance of gingival hyperplasia with time in our patients.

83 Digestive disorders are a known complication of *KCNH1*-related encephalopa-  
84 thy, constipation and GERD affecting 60% and 25% of the patients respectively  
85 (Table S1). Though these comorbidities are not surprising in a context of severe  
86 encephalopathy, their severity in some of our patients is noteworthy.

87 A previous study reported four patients with *KCNH1*-related encephalopathy out  
88 of 1447 with ID (2.7‰).[4] We found three French patients out of 2450 studied  
89 on an epilepsy gene panel and three by WES out of 2144 with ID (total 6/4594).  
90 We conclude that the prevalence of the *KCNH1*-related encephalopathy in indi-  
91 viduals with epilepsy/ID and without features of TBS/ZLS in France is about  
92 1.3‰.

93

94 Three unrelated index cases of our series were heterozygous for the  
95 c.1486G>A p.(Gly496Arg) variant. This variant and the c.1487G>A  
96 (p.Gly496Glu) substitution affecting the same Glycine residue have already

97 been reported in two patients with *KCNH1*-related encephalopathy.[7, 15, 16]  
98 Interestingly, both patients also had mild extra-neurological phenotypes: the  
99 individual carrying p.(Gly496Arg) did not have nail hypo/aplasia (but gingival  
100 enlargement and moderate hypertrichosis),[7, 15] and the other with  
101 p.(Gly496Glu) only presented broad thumbs/toes but no nail aplasia, hypertri-  
102 chosis, or gingival enlargement.[16] Since these patients are the only published  
103 cases of *KCNH1*-related encephalopathy lacking these key features of  
104 TBS/ZLS, this raises the question of a potential genotype-phenotype correlation  
105 associating mild or absent extra-neurological signs and variants affecting the  
106 Gly496 residue.

107 Functionally tested variants in *KCNH1* usually lead to a left-ward shift in voltage  
108 dependent activation, producing an increase in K<sup>+</sup> conductance in the negative  
109 potential-range (or a so called gain-of-function effect).[7] These variants may  
110 then contribute, as suggested by Kortum et al., to a functional blockade of volt-  
111 age gated Na<sup>+</sup> and L-type Ca<sup>2+</sup> channels through stabilization of the membrane  
112 potential to a more negative voltage range, and draw parallels with the gingival  
113 hyperplasia that can be seen with prolonged use of the Na<sup>+</sup> channel blocker  
114 phenytoin or the Ca<sup>2+</sup> channel blocker nifedipine.[7] *KCNH1* is also required for  
115 resorption of the primary cilium when the cell cycle is reinitiated: hyperactivation  
116 of *KCNH1* was indeed shown to increase resorption. Alterations of the sonic  
117 hedgehog (SHH) cilia-mediated pathway involved in morphogenesis were there-  
118 fore proposed to lead to the skeletal and nail malformations.[17, 20]

119

120 When functionally tested in a heterologous cell system, the homotetrameric  
121 p.(Gly496Arg) mutant *KCNH1* channel failed to produce depolarization-  
122 activated currents demonstrating a loss of function effect. When the  
123 p.(Gly496Arg) mutant was co-expressed with wild-type *KCNH1* to produce het-  
124 erotetrameric channels, an increase in K<sup>+</sup> conductance was seen at negative  
125 membrane potentials.[7] In addition, similarly to other studied pathogenic vari-  
126 ants, significant reduced K<sup>+</sup> conductance was reported at depolarizing poten-  
127 tials.[7]

128 It is tempting to hypothesize that the absence or subtlety of extra-neurological  
129 symptoms in patients with specific variants such as p.(Gly496Arg) compared to  
130 variants associated with a “classical” ZLS/TBS phenotype is reflecting tissue-  
131 specific sensitivities to a more limited gain-of-function effect of this variant. More  
132 in-depth comparative studies of the effect of specific *KCNH1* variants on SHH  
133 pathway and voltage-gated calcium channel activity is warranted, preferably in  
134 model systems recapitulating the human phenotype, such as human induced  
135 pluripotent stem cell derived neuronal and chondroprogenitor cells.

136

137

138 Two of our novel variants, c.1475C>A p.(Ala492Asp) and c.1478C>A  
139 p.(Thr493Asn), are located in the S6 transmembrane of Kv10.1 (Figure 1B)  
140 where other variants [c.1480A>G p.(Ile494Val) and the c.1465C>T  
141 p.(Leu489Phe)] have been previously reported in typical ZLS/TBS patients.[4, 7,  
142 15] The c.881C>T p.(Thr294Met) variant is for its part located in the S3 trans-  
143 membrane domain. The c.2078G>A p.(Arg693Gln) variant is the first reported to

144 affect a residue in the C-terminal cyclic nucleotide binding homology domain  
145 (CNBHD). The biochemical and electrophysiological consequences of this  
146 change have to be determined, but could impact the interaction between the  
147 *ether-a-go-go* domain and the CNBHD, leading to an impairment of the activa-  
148 tion/deactivation channel control.[21]

149 To further investigate the potential genotype-phenotype correlations in *KCNH1*-  
150 related encephalopathy, the description of further thoroughly phenotyped cases  
151 will be necessary. Meanwhile, the possibility of non-syndromic *KCNH1*-related  
152 encephalopathies should be considered when designing epilepsy gene panels  
153 or interpreting whole exome data from individuals with epilepsy or non-specific  
154 ID.

155

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162 **CONFLICT OF INTEREST**

163 The authors declare no conflict of interest.

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166 **ETHICS APPROVAL**

167 This study was designed in compliance with the tenets of the Helsinki Declara-  
168 tion.

169 **PATIENT CONSENT**

170 Written consents for genetic testing and publication were obtained locally from  
171 all participants or their representatives.

172 **AUTHOR CONTRIBUTIONS**

173 MAM/OP: Data collection, Data analysis, Manuscript writing, Manuscript editing

174 SWh/SWe: Data collection, Manuscript writing, Manuscript editing

175 LA/DD/CK/SK/ELG/GL/CN/SV/LV/BK/BC/MN: Data collection, Manuscript edit-  
176 ing

177 GB/JB/AP: Data collection

178 CM: Study conception, Data collection, Data analysis, Manuscript writing, Man-  
179 uscript editing

180 All authors read and approved the final manuscript.



181 **Figure 1. Schematic representation of all (likely) pathogenic variants identified**  
182 **in *KCNH1*.**

183 **A. cDNA level:** Legend: in bold, the novel variants reported here.

184 **B. protein level:** Legend: Stars representing variants identified, in blue = variants  
185 identified in our series, Sn = transmembrane domains, EAG = *Ether-à-go-go*  
186 domain, CNBHD = cyclic nucleotide binding homology domain, P = Pore-  
187 lining loop.

188

189 **Figure S1. Faces, hands and feet of patients P2, P4, P5, and P7.**

190 Mild dysmorphic features could be described: long facies with high forehead,  
191 bilateral epicanthic folds, mild hypertelorism, flat nasal bridge, broad tip of nose,  
192 flat midface, full lips, small mouth, mild prognathism.

193

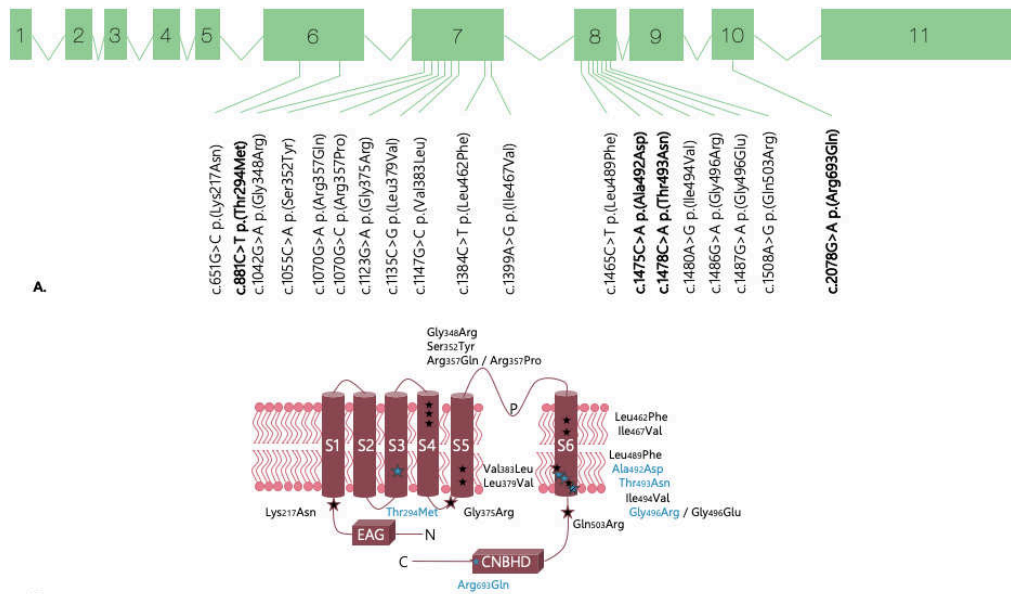
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**Figure 1: Schematic representation of all (likely) pathogenic variants identified in *KCNH1*.**

**A. cDNA level:** in bold, the novel variants reported here.

**B. protein level:** Legend: Stars representing variants identified, In blue = variants identified in our series, Sn = transmembrane domains, EAG = *Ether-à-go-go* domain, CNBHD = cyclic nucleotide binding homology domain, P = Pore-lining loop.

## Supplementary methods

278

279

280 The DNA of patient 1 (P1) was analyzed on a 116 epilepsy gene panel. Next-  
281 generation sequencing was performed using a SureSelectXT Custom 12-24Mb library  
282 (Agilent Technologies) on a ion proton platform (Thermo Fisher). The results were ana-  
283 lyzed using the Torrent suite (Thermo Fisher) for alignment and variant calling. To filter  
284 and establish coverage and variant analysis, we used the Varaft annotation and filtra-  
285 tion system (Desvignes et al., Nucleic Acids Res. 2018 Jul 2; 46: W545–W553.doi:  
286 10.1093/nar/gky471)

287

288 The DNA of patient 4 (P4) and patient 5 (P5) was analyzed on a 90 epilepsy gene pan-  
289 el. A library of all coding exons and intron-exon boundaries was prepared using a Se-  
290 qCap EZ Library (Roche-NimbleGen) following the manufacturer's instructions. Gene-  
291 panel sequencing was performed on a MiSeq (Illumina Inc). Sequence alignment was  
292 performed by Genodiag with BWA 0.7.12, picard-tools-1.121, GATK-3.5 (Indel realign-  
293 ment, base recalibration). GATK-3.5 (HaplotypeCaller) and SNPEff-4.2 were used for  
294 variant calling and annotation, adding information from gnomAD, ClinVar and HGMD.

295

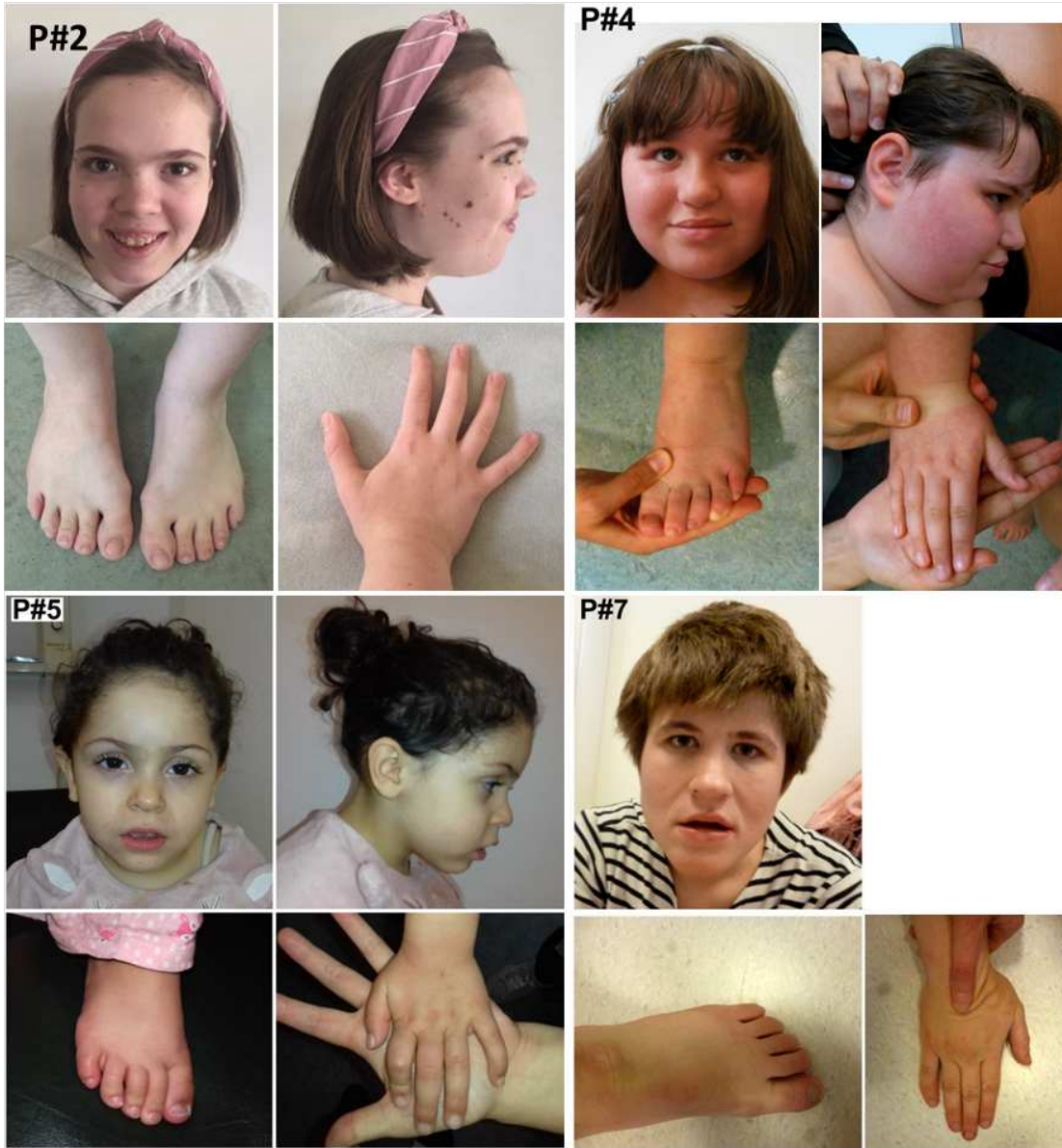
296 The DNA of patients 2 (P2), 3 (P3), 6 (P6) and 7 (P7) and their parents was studied by  
297 trio-WES. A library of all coding exons and intron-exon boundaries was prepared using  
298 a SeqCap EZ MedExome (Roche-NimbleGen) following the manufacturer's instruc-  
299 tions. Whole Exome sequencing was performed on the Illumina NextSeq 500 platform.

300 P2, P3 and P6: Raw reads were mapped to the human genome reference-build hg19  
301 using the Burrows Wheeler Aligner (BWA MEM v0.717) alignment algorithm. The re-  
302 sulting binary alignment/map (BAM) files were further processed by Genome Analysis  
303 Tool Kit HaplotypeCaller (GATK HC v3.8). The VCF files were then annotated on  
304 Snpeff version 4.3T. Only coding non synonymous and splicing variants were consid-

305 ered. Variant prioritization was conducted thanks to the transmission mode (*de novo*,  
306 autosomal recessive and X-linked), and the frequency of the variants in the gnomAD  
307 database.

308 P7: Fastq files were aligned to human genome hg19 with bwa mem (v0.7.3). We then  
309 called SNVs and INDELs following GATKs best practices (v3.4). We achieved an aver-  
310 age mean target coverage of 113X. Variants were annotated using ANNOVAR and  
311 filtered with in-house scripts to keep variants with at least 9 reads and with a variant  
312 read frequency over 20 percent impacting exonic sequences or splice sites (+/- 10bp  
313 from the junction) and with an allele frequency <0.5% in 1000 genomes, genome ag-  
314 gregation database (gnomAD, 123,136 exomes and 15,496 whole genome sequences;  
315 accessed on 11/10/2018) and in a local database. The possible functional impact of  
316 amino-acid changes was predicted by SIFT (Sorting Intolerant from Tolerant), Poly-  
317 Phen-2 hvar and CADD score (Combined Annotation Dependent Depletion). The Ala-  
318 mut software (Interactive biosoftware) was used to study retained variant sites.

319



**Figure S1. Faces, hands and feet of patients P2, P4, P5, and P7.**  
 Mild dysmorphic features could be described: long facies with high forehead, bilateral epicanthic folds, mild hypertelorism, flat nasal bridge, broad tip of nose, flat midface, full lips, small mouth, mild prognathism.