Exome sequencing findings in 27 patients with myoclonic-atonic epilepsy: Is there a major genetic factor?

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Abstract

Myoclonic-atonic epilepsy (MAE) is thought to have a genetic etiology. Mutations in *CHD2*, *SLC2A1* and *SLC6A1* genes have been reported in few patients showing often intellectual disability prior to MAE onset. We aimed to explore putative causal genetic factors in MAE. We performed array-CGH and whole-exome sequencing in 27 patients. We considered non-synonymous variants, splice acceptor, donor site mutations, and coding insertions/deletions. A gene was causal when its mutations have been already linked to epilepsy or other brain diseases or when it has a putative function in neuronal excitability or brain development. We identified candidate disease-causing variants in 11 patients (41%). Single variants were found in some known epilepsy-associated genes (namely *CHD2*, *KCNT1*, *KCNA2* and *STXBP1*) but not in others (*SLC2A1* and *SLC6A1*). One new candidate gene *SUN1* requires further validation. MAE shows underlying genetic heterogeneity with only few cases linked to mutations in genes reported in developmental and epileptic encephalopathies.

KEYWORDS

Doose syndrome, epileptic encephalopathy, exome sequencing, genes, myoclonic-atonic epilepsy

1 | INTRODUCTION

Myoclonic-atonic epilepsy (MAE) is a rare epileptic encephalopathy estimated to account for 1% to 2% of childhood-onset epilepsies.^{1,2}

MAE is diagnosed in a child with normal development, with onset of myoclonic, myoclonic-atonic or atonic seizures between 7 months and 6 years old and generalized spike or polyspike-wave discharges on the electroencephalogram (EEG).³

The cause of MAE has yet to be determined. However, high incidence of epilepsy in patients' family^{1,2,4,5} and a higher prevalence among patients' relatives (200 times higher than in the general population)² suggest a genetic basis. Some genes have been linked to sporadic cases of MAE: *SLC2A1*, *SLC6A1*, *CHD2*, *SCN1A*, *GABRG2*,

SCN2A, *CACNA1H*, *SYNGAP1* and *SCN1B*.^{4,6-14} Many of the patients presented a pre-existing intellectual disability (ID) therefore showing atypical clinical presentation¹⁵ (Table 1). To identify possible major genetic factors, we performed array-comparative genomic hybridization (CGH) and whole-exome sequencing (WES) in 27 patients with typical MAE.

2 | MATERIALS AND METHODS

2.1 | Patients

We included 22 males and 5 females with MAE, without structural brain lesion or underlying metabolic diseases. Age of onset ranged

TABLE 1 Clinical and electroencephalographic features of previously reported MAE cohorts with an identified mutation

Gene	Number of patients with the mutation	Age at onset of seizures	Development before the onset of epilepsy	Electroencephalographic main features	Authors
<i>SLC2A1</i>	4	26.6 months (8-48)	Delayed for 3 of the 4	Typical: generalized SW or PSW	Mullen et al. 2011 ¹²
<i>SLC6A1</i>	7	24.7 months (12-36)	Delayed for all 7	Atypical: SW on eye closure, PPR, continuous SW discharges during sleep, atypical absences, hyperventilation-provoked subclinical paroxysms Typical: generalized SW or PSW, slow background activity	Carvill et al. 2015 ⁴
<i>CHD2</i>	1	41 months	Normal	Atypical: PPR Typical: generalized SW wave, slow background activity	Trivisano et al. 2015 ⁶
<i>SCN1A</i>	1	36 months	Normal	Typical: generalized PSW	Dimova and Kietzmann 2010 ¹⁶
	1	18 months	Normal	Atypical: PPR Typical: generalized SW, slow background activity	Yordanova et al. 2011 ¹¹
<i>GABRG2</i>	1	Not reported	Not reported	Not reported	Wallace et al. 2001 ⁸
<i>CACNA1H</i>	4	Not reported	Not reported	Not reported	Heron et al. 2007 ⁹
<i>SCN1B</i>	1	Not reported	Not reported	Not reported	Wallace et al. 1998 ⁷
<i>SCN2A</i>	2	15 and 24 months	Delayed in one and Normal in the other	Typical: generalized SW, slow background activity	Wolff et al. 2017 ¹⁴
<i>SYNGAP1</i>	3 (MAE like)	22, 24 and < 24 months	Delayed for all 3	Atypical: PPR, focal spike wave Typical: generalized SW and PSW	Mignot et al. 2016 ¹³

Abbreviations: MAE, myoclonic-atonic epilepsy; PPR, photoparoxysmal response, PSW, polyspike-waves, SW, spike-waves.

from 1.5 to 5.6 years. All patients had at least 5 years follow-up after the onset. Patients' legal guardians provided written informed consent. The Cochin-Port-Royal Hospital Investigational Review Board (France) approved the study.

2.2 | Array-CGH and WES

Genomic DNA was extracted from peripheral blood using a standard procedure. We first performed array-CGH by 60 K oligonucleotide microarray (Agilent Technologies, Santa Clara, California) according to the manufacturer's recommendations.

Exome capture was performed by the SureSelectXT Human All Exon V3 50 Mb target-enrichment kit (Agilent Technologies, Massy, France) in trios (proband and parents). Sequencing was performed using a paired-end strategy and an average read length of 75 bases and average coverage of 130x (Illumina HISEQ, Illumina Inc., San Diego, California). Sequences were aligned to the human genome reference sequence (hg19 assembly) with the Burrows-WheelerAligner (Illumina).

Downstream processing was carried out with the Genome Analysis Toolkit (GATK),¹⁷ SAMtools¹⁸ and Picard Tools. An in-house pipeline performed genetic variation annotation.

2.3 | WES analysis

Trio analysis was performed considering all Mendelian inheritance models. We excluded common genetic variants already described in public or in-house datasets with allelic frequencies >0.01. We focused on nonsynonymous variants, splice acceptor and donor site variants or coding indels. Sanger sequencing confirmed all retained variants.

3 | RESULTS

Two patients (7 and 10) had a family history of febrile seizures that resolved by the age of 5 years with no further epilepsy, neurologic or psychiatric disorders. Array-CGH showed a 575-kb de novo duplication in 5q22.3 in one patient with no relevant epilepsies genes included in this interval. He showed moderate ID during follow-up.

WES highlighted a candidate disease-causing gene in 11 patients (41%). Five patients (19%) had mutations in epilepsy genes but not the other six (22%). 36% were de novo, 18% X-linked and 36% showed recessive inheritance (Table 2). We classified these mutations as causal, highly probable or probable candidates (Table 3).

TABLE 2 Identified, selected mutations and inheritance using whole-exome sequencing (WES) in our cohort

Proband	Sex	CGH	Gene	Inheritance	Mutation	Previous descriptions in public databases	PolyPhen-2 (HumVar)	SIFT	Mutation taster
Genes already reported in patients with MAE									
7	F	N	<i>CHD2</i> ^a	de novo	NM_001271.3(CHD2): c.1861C > T; p. Arg621Trp	—	Probably damaging (1)	Deleterious (score: 0)	Disease-causing (P-value: 1)
10	M	N	<i>CACNA1H</i>	CH	NM_021098.2: c.3283G > A; p. Asp1095Asn	ExAC	Probably damaging (Score: 1)	Deleterious (0.03)	Disease-causing (P-value: .955)
					NM_021098.2: c.4756A > G; p. Arg1586Gly	ExAC and Variants dbSNP	Probably damaging (Score: 0,979)	Deleterious (0)	Disease-causing (P-value: .984)
Genes already reported in other epilepsies and in Developmental epileptic encephalopathies									
5	F	N	<i>KCNA2</i>	de novo	NM_004974.3: c.889C > T; p. Arg297Trp	—	Probably damaging (score: 1)	Deleterious (score: 0)	Disease-causing (P-value: 1)
8	M	N	<i>STXBP1</i> ^a	de novo	NM_003165.3: c.288 T > C; UTR		UTR	UTR	UTR
6	M	N	<i>KCNT1</i>	de novo	NM_020822.2: c.862G > A; p. Gly288Ser	ClinVar	Probably damaging (score: 0.978)	Tolerated (score: 0.26)	Disease-causing (P-value: 1)
9	M	N	<i>MECP2</i> ^a	MI	NM_001110792.1: c.259C > A; p.Pro87Thr	—	Possibly damaging (0,794)	Deleterious (score: 0)	Disease-causing (P-value: 1)
Genes reported in neurodevelopmental disorders									
3	M	N	<i>SHROOM4</i>	Not tested	NM_020717.3: c.1201C > T; p. His401Tyr	Variants dbSNP, ExAC	Benign (0,410)	Deleterious (0.05)	Disease-causing (P-value: .589)
1	M	N	<i>FTSJ1</i> ^a	MI	NM_012280.3(FTSJ1): c. ^a 9+ 1G > T	—	Essential splicing	Essential splicing	Essential splicing
4	M	N	<i>KLC2</i>	CH	NM_001134775.1: c.1295G > A; p. Gly432Glu	ExAC and Variants dbSNP	Probably damaging (1)	Tolerated (0.13)	Disease-causing (P-value: 1)
					NM_001134775.1: c.1785 + 42G > T	—	Intronic	Intronic	Intronic
2	F	N	<i>TPP1</i> ^a	CH	NM_000391.3(TPP1): c.509-1G>	—	Essential splicing	Essential splicing	Essential splicing
					NM_000391.3(TPP1): c.622C > T; p.Arg208 ^a	ExAC, ClinVar, COSMIC, ESP	Stop-gained	Stop-gained	Stop-gained
Gene not reported in neurological diseases									
11	M	N	<i>SUN1</i> ^a	CH	NM_001130965:c.1779 + 2_1779 + 9dup; p.?	—	Splicing frameshift	Splicing frameshift	Splicing frameshift
					NM_001130965: c.956C > G; p. Pro319Arg	—	Probably damaging (Score: 1)	Tolerated (0.15)	Disease-causing (P-value: 1)

Abbreviations: CH, compound heterozygous; F, female; M, male; MI, maternally inherited; N, normal; UTR, untranslated transcribed region.

^aAutism spectrum disorder.

3.1 | Genes already reported in MAE

A de novo *CHD2* missense variant (NM_001271.3:c.1861C > T;p. Arg621Trp) was identified in Patient 7. Proband 10 had compound heterozygous variants in *CACNA1H* (NM_021098.2:c.3283G > A;p. Asp1095Asn and.

NM_021098.2:c.4756A > G;p.Arg1586Gly).

3.2 | Genes reported in other epilepsies

A de novo missense *KCNA2* variant (NM_004974.3:c.889C > T; p.Arg297Trp) was found in Patient 5. Another mutation affecting the same codon (c.890G > A;p.Arg297Gln) had already been linked to developmental and epileptic encephalopathies (DEE).³³

TABLE 3 Selection of variants as pathogenic or potentially damaging

Gene	Reported in MAE	Reported in other epilepsies	Associated with neurodevelopmental disorders	Proteins expressed in brain/reported in animal model	Function of the encoded protein	Described neurological pathologies	Known IM	Suspected IM in our cohort
<i>CHD2</i>	Carvill et al. 2013, ¹⁹ Trivisano et al. 2015 ⁶	Carvill et al. 2013, ¹⁹ Trivisano et al. 2015 ⁶	Carvill et al. 2013, ¹⁹ Trivisano et al. 2015 ⁶	Trivisano et al. 2015 ⁶	DNA recombination and cell-cycle regulation	atypical DS, LGS, Jeavons syndrome and MAE	AD	AD
<i>CACNA1H</i>	Heron et al. 2007 ⁹	Heron et al. 2007 ⁹	Heron et al. 2007, ⁹ Splawski et al. 2006 ²⁰	Heron et al. 2007, ⁹ Splawski et al. 2006 ²⁰	T-type calcium channel	absence epilepsy, idiopathic generalized epilepsy, febrile seizures and MAE	AD	AR
<i>KCNA2</i>	—	Syrbe et al. 2015 ²¹	Syrbe et al. 2015 ²¹	Syrbe et al. 2015 ²¹	Voltage-gated potassium channel	early childhood onset EE	AD	AD
<i>STXBP1</i>	—	Saitsu et al. 2008, ²² Stamberger et al. 2016 ²³	Saitsu et al. 2008, ²² Stamberger et al. 2016 ²³	Saitsu et al. 2008, ²² Stamberger et al. 2016 ²³	Regulation of synaptic vesicle docking and fusion	EE, infantile spasms, LGS, ID and fever sensitive epilepsies	AD	AD
<i>KCNT1</i>	—	Barcia et al. 2012 ²⁴	Barcia et al. 2012 ²⁴	Barcia et al. 2012 ²⁴	Sodium-activated potassium channel	EIMFS and nocturnal frontal lobe epilepsy	AD	AD
<i>MECP2</i>	—	Villard 2007 ²⁵	Villard 2007 ²⁵	Villard 2007 ²⁵	Connection and synaptic regulation	Rett syndrome and severe ID	X-linked	X-linked
<i>SHROOM4</i>	—	—	Hagens et al. 2006, ²⁶ Yoder and Hildebrand 2007 ²⁷	Hagens et al. 2006, ²⁶ Yoder and Hildebrand 2007 ²⁷	Cytoskeletal architecture regulation	X-linked ID	X-linked	—
<i>FTSJ1</i>	—	—	Ramser et al. 2004, ²⁸ Freude et al. 2004 ²⁹	Ramser et al. 2004, ²⁸ Freude et al. 2004 ²⁹	Supposed translation regulation	X-linked ID	X-linked	X-linked
<i>TPP1</i>	—	—	Sun et al. 2013 ³⁰	Sun et al. 2013 ³⁰	Lysosomal serine protease	ceroid lipofuscinosis 2 and spinocerebellar ataxia	AR	AR
<i>KLC2</i>	—	—	—	Melo et al. 2015 ³¹	Anterograde axoplasmatic transport	spastic paraplegia, optic atrophy and neuropathy	AR	AR
<i>SUN1</i>	—	—	—	Zhang et al. 2009 ³²	Neurogenesis and interkinetic nuclear migration	—	—	—

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; DS, Dravet syndrome; EE, epileptic encephalopathies; EIMFS, epilepsy of infancy with migrating focal seizures; ID, intellectual disability; IM, inheritance mode; LGS, Lennox-Gastaut syndrome; MAE, myoclonic-atonic epilepsy.

Proband 8 carried a de novo variant in *STXBP1* (NM_003165.3: c.288 T > C).

A de novo *KCNT1* mutation (NM_020822.2:c.862G > A;p.Gly288-Ser) previously described in epilepsy of infancy with migrating focal seizures (EIMFS) and in autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) was found in proband 6.^{24,34}

Lastly, proband 9 showed a maternally inherited *MECP2* variant (NM_001110792.1:c.259C > A;p.Pro87Thr) that alters a conserved amino acid.

3.3 | Genes reported in neurodevelopmental disorders

Four strong candidate genes have already been linked to autism spectrum disorder (ASD), ID and neurodegenerative brain disease (Table 3). Variants in *SHROOM4* (NM_020717.3:c.1201C > T; p.His401Tyr) and *FTSJ1* (NM_012280.3:c.*9 + 1G > T) were identified. A missense mutation (NM_001134775.1:c.1295G > A;p.Gly432Glu) was combined with an intronic mutation (patient 4) (NM_001134775.1;c.1785 + 42G > T) in *KLC2*.

Patient 2 had a nonsense mutation (NM_000391.3:c.622C > T;p.Arg208*) and a splice variant (NM_000391.3:c.509-1G>) in *TPP1* (already linked to ceroid lipofuscinosis 2 and autosomal recessive spinocerebellar ataxia).^{30,35}

3.4 | Probable candidate gene not reported in known central nervous system diseases

Proband 11 showed a compound heterozygous variants in *SUN1*, with a missense mutation (NM_001130965:c.956C > G;p.Pro319Arg) and a splice variant (NM_001130965:c.1779 + 2_1779 + 9dup). *SUN1* is involved in neurogenesis, nuclear connections and interkinetic migration.³⁵

3.5 | ID and ASD during follow-up

All patients (1-11) with reported genes showed moderate ID during follow-up. ASD was present in six patients (Table 2). In patients with no reported mutations, ID was reported in 25% and ASD in 15%.

4 | DISCUSSION

The present study reports WES results in a cohort of MAE. We found mutations in epilepsy genes in only 19% of cases but we did not replicate the high prevalence of mutations in *SLC2A1* or *SLC6A1*.^{4,12} Our results highlighted the genetic heterogeneity of MAE phenotype and extended it to causal genes in DEE.

We found pathogenic variants in two genes previously linked to MAE. *CHD2* is reported in MAE, atypical Dravet syndrome (DS), Lennox-Gastaut syndrome (LGS) and Jeavons syndrome.^{6,15,19} Patients present common characteristics as previous developmental delay, fever and photosensitivity. Myoclonic seizures and ASD are

often reported.^{6,15} Our patient did not present these characteristics extending the phenotypic spectrum of *CHD2* mutations to typical MAE.

Mutations in *CACNA1H* were described in a few patients with MAE,⁹ but also in patients with ASD and generalized epilepsy.^{9,20} The co-existence of two compound heterozygous variants in patient 10 might suggest that *CACNA1H* is causal.

We extended the spectrum of genes reported in epilepsies and mainly in DEE to typical MAE phenotype as *KCNA2*, *STXBP1* and *KCNT1*.^{22,23,36} The mutation reported in *KCNT1* was already reported in both major phenotypes, EIFMS and ADNFLE.^{24,34} A few patients were reported with Ohtahara syndrome. Mutations in *MECP2* in males are responsible for severe ID²⁵ with rarely reported epilepsy. The normal development still the age of 18 months could question the causality of this mutation, however, the known phenotype spectrum of many developmental genes and the pathogenic nature of the variant allowed us to hypothesize a causal effect in this patient.

Lastly, we identified the genes *SHROOM4*, *FTSJ1*, *TPP1*, *KLC2* and *SUN1* as strong new candidates. *SHROOM4* and *FTSJ1* mutations have been reported in neurological impairments, including X-linked ID.^{26,28} *KLC2* mutations have been reported in neurodegenerative disorder combining spastic paraplegia, optic atrophy and neuropathy.^{31,35}

We had difficulty analyzing significant variants in *SUN1* expressed in the nervous system and not previously reported in brain diseases. Its involvement in MAE needs to be confirmed by further case studies and functional investigations.

Finally, all patients with epilepsy genes or genes reported in neurodevelopmental disorders showed ID and some presented later ASD. This higher incidence of ID and ASD compared to the group with no identified genes might be related to the direct developmental impact of these genes beyond seizures and EEG impact. The normal development before the epilepsy onset is interesting in this cohort and is similar to a few patients reported with DEE genes and a normal neurological development before epilepsy onset.

This study reaffirms that copy number variation accounts for a small proportion of MAE as for Genetic (idiopathic) generalized epilepsies.^{37,38} MAE might be considered as a heterogeneous condition as infantile spasms or LGS with few monogenic cases and with others possible multigenic, epigenetic and/or environmental factors involved in the emergence of MAE.

The identification of rare but treatable etiologies as Glut1 deficiency is to be emphasized although we did not find this mutation in our series. The lack of a major causal gene raises major questions on the homogeneity of this electro-clinical phenotype with in some reports the use of "MAE-like" phenotype.

Finally, our data emphasize the difficulty to establish the causal role of variants even in homogeneous cohorts. The larger use of WES that is increasingly replacing gene panels should help accumulating data from phenotyped cohorts to broaden the spectrum of known genes and helping to discover new causal genes.

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CONFLICT OF INTEREST

We declare no conflict of interest.

DATA ACCESSIBILITY

Data available on request from the authors

REFERENCES

1. Tang S, Pal DK. Dissecting the genetic basis of myoclonic-astatic epilepsy. *Epilepsia*. 2012;53:1303-1313.
2. Kelley SA, Kossoff EH. Dooze syndrome (myoclonic-astatic epilepsy): 40 years of progress. *Dev Med Child Neurol*. 2010;52:988-993.
3. Proposal for revised classification of epilepsies and epileptic syndromes. Commission on classification and terminology of the international league against epilepsy. *Epilepsia*. 1989;30:389-399.
4. Carvill GL, McMahon JM, Schneider A, et al. Mutations in the GABA transporter SLC6A1 cause epilepsy with myoclonic-atonic seizures. *Am J Hum Genet*. 2015;96:808-815.
5. Kaminska A, Ickowicz A, Plouin P, Bru MF, Dellatolas G, Dulac O. Delineation of cryptogenic Lennox-Gastaut syndrome and myoclonic atstatic epilepsy using multiple correspondence analysis. *Epilepsy Res*. 1999;36:15-29.
6. Trivisano M, Striano P, Sartorelli J, et al. CHD2 mutations are a rare cause of generalized epilepsy with myoclonic-atonic seizures. *Epilepsy Behav*. 2015;51:53-56.
7. Wallace RH, Wang DW, Singh R, et al. Febrile seizures and generalized epilepsy associated with a mutation in the Na⁺-channel beta1 subunit gene SCN1B. *Nat Genet*. 1998;19:366-370.
8. Wallace RH, Marini C, Petrou S, et al. Mutant GABA(a) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. *Nat Genet*. 2001;28:49-52.
9. Heron SE, Khosravani H, Varela D, et al. Extended spectrum of idiopathic generalized epilepsies associated with CACNA1H functional variants. *Ann Neurol*. 2007;62:560-568.
10. Dimova PS, Yordanova I, Bojinova V, Jordanova A, Kremenski I. Generalized epilepsy with febrile seizures plus: novel SCN1A mutation. *Pediatr Neurol*. 2010;42:137-140.
11. Yordanova I, Todorov T, Dimova P, et al. One novel Dravet syndrome causing mutation and one recurrent MAE causing mutation in SCN1A gene. *Neurosci Lett*. 2011;494:180-183.
12. Mullen SA, Marini C, Suls A, et al. Glucose transporter 1 deficiency as a treatable cause of myoclonic atstatic epilepsy. *Arch Neurol*. 2011;68:1152-1155.
13. Mignot C, von Stulpnagel C, Nava C, et al. Genetic and neurodevelopmental spectrum of SYNGAP1-associated intellectual disability and epilepsy. *J Med Genet*. 2016;53:511-522.
14. Wolff M, Johannesen KM, Hedrich UBS, et al. Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders. *Brain*. 2017;140:1316-1336.
15. Carvill G, Helbig I, Mefford H. CHD2-related neurodevelopmental disorders. In: Pagon RA, Adam MP, Ardinger HH, et al., eds. Seattle, WA: GeneReviews; 1993.
16. Dimova EY, Kietzmann T. Hypoxia-inducible factors: post-translational crosstalk of signaling pathways. *Methods Mol Biol*. 2010; 647:215-236.
17. McKenna A, Hanna M, Banks E, et al. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;20:1297-1303.
18. Li H, Handsaker B, Wysoker A, et al. 1000 Genome Project Data Processing Subgroup. The sequence alignment/map format and SAMtools. *Bioinformatics*. 2009;25:2078-2079.
19. Carvill GL, Heavin SB, Yendle SC, et al. Targeted resequencing in epileptic encephalopathies identifies de novo mutations in CHD2 and SYNGAP1. *Nat Genet*. 2013;45:825-830.
20. Splawski I, Yoo DS, Stotz SC, Cherry A, Clapham DE, Keating MT. CACNA1H mutations in autism spectrum disorders. *J Biol Chem*. 2006;281:22085-22091.
21. Syrbe S, Hedrich UB, Riesch E, et al. De novo loss- or gain-of-function mutations in KCNA2 cause epileptic encephalopathy. *Nat Genet*. 2015;47:393-399.
22. Saito H, Kato M, Mizuguchi T, et al. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. *Nat Genet*. 2008;40:782-788.
23. Stamberger H, Nikanorova M, Willemsen MH, et al. STXBP1 encephalopathy: a neurodevelopmental disorder including epilepsy. *Neurology*. 2016;86:954-962.
24. Barcia G, Fleming MR, Deligniere A, et al. De novo gain-of-function KCNT1 channel mutations cause malignant migrating partial seizures of infancy. *Nat Genet*. 2012;44:1255-1259.
25. Villard L. MECP2 mutations in males. *J Med Genet*. 2007;44:417-423.
26. Hagens O, Dubos A, Abidi F, et al. Disruptions of the novel KIAA1202 gene are associated with X-linked mental retardation. *Hum Genet*. 2006;118:578-590.
27. Yoder M, Hildebrand JD. Shroom4 (Kiaa1202) is an actin-associated protein implicated in cytoskeletal organization. *Cell Motil Cytoskeleton*. 2007;64:49-63.
28. Ramser J, Winnepeninckx B, Lenski C, et al. A splice site mutation in the methyltransferase gene FTSJ1 in Xp11.23 is associated with non-syndromic mental retardation in a large Belgian family (MRX9). *J Med Genet*. 2004;41:679-683.
29. Freude K, Hoffmann K, Jensen LR, et al. Mutations in the FTSJ1 gene coding for a novel S-adenosylmethionine-binding protein cause non-syndromic X-linked mental retardation. *Am J Hum Genet*. 2004;75:305-309.
30. Sun Y, Almomeni R, Breedveld GJ, et al. Autosomal recessive spinocerebellar ataxia 7 (SCAR7) is caused by variants in TPP1, the gene involved in classic late-infantile neuronal ceroid lipofuscinosis 2 disease (CLN2 disease). *Hum Mutat*. 2013;34:706-713.
31. Melo US, Macedo-Souza LI, Figueiredo T, et al. Overexpression of KLC2 due to a homozygous deletion in the non-coding region causes SPOAN syndrome. *Hum Mol Genet*. 2015;24:6877-6885.
32. Zhang X, Lei K, Yuan X, et al. SUN1/2 and Syne/Nesprin-1/2 complexes connect centrosome to the nucleus during neurogenesis and neuronal migration in mice. *Neuron*. 2009;64:173-187.
33. Pena SDJ, Coimbra RLM. Ataxia and myoclonic epilepsy due to a heterozygous new mutation in KCNA2: proposal for a new channelopathy. *Clin Genet*. 2015;87:e1-e3.
34. Ishii A, Shioda M, Okumura A, et al. A recurrent KCNT1 mutation in two sporadic cases with malignant migrating partial seizures in infancy. *Gene*. 2013;531:467-471.
35. Elleder M, Dvorakova L, Stolnaja L, et al. Atypical CLN2 with later onset and prolonged course: a neuropathologic study showing different sensitivity of neuronal subpopulations to TPP1 deficiency. *Acta Neuropathol*. 2008;116:119-124.

36. Hamdan FF, Piton A, Gauthier J, et al. De novo STXBP1 mutations in mental retardation and nonsyndromic epilepsy. *Ann Neurol*. 2009;65:748-753.
37. Epilepsy Phenome/Genome Project Epi KC. Copy number variant analysis from exome data in 349 patients with epileptic encephalopathy. *Ann Neurol*. 2015;78:323-328.
38. Mullen SA, Berkovic SF, the ILAE Genetics Commission. Genetic generalized epilepsies. *Epilepsia*. 2018;59:1148-1153.