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

Laura Routier<sup>1,2,3</sup>, Florine Verny<sup>1,4</sup>, Giulia Barcia<sup>5</sup>, Nicole Chemaly<sup>1,4</sup>, Isabelle Desguerre<sup>1</sup>, Laurence Colleaux<sup>4</sup>, Rima Nabbout<sup>1,4</sup>

<sup>1</sup>Reference Centre for Rare Epilepsies, Pediatric Neurology, Necker Enfants-Malades Hospital, Paris, France

<sup>2</sup>Pediatric Neurology, Amiens-Picardie University Hospital, Amiens, France

<sup>3</sup>GRAMFC-INSERM U1105, UPJV, Amiens, France

<sup>4</sup>INSERM UMR1163, Translational Research for Neurological Disorders, Imagine Institute, Paris-Descartes University, Paris, France

<sup>5</sup>Clinical Genetics, Necker Enfants-Malades Hospital, Paris, France

## Correspondence

Reference Centre for Rare Epilepsies, Pediatric Neurology, Necker Enfants-Malades Hospital, Paris, France.

Email: routier.laura@chu-amiens.fr

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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.<sup>1,2</sup>

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).<sup>3</sup>

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

*SCN2A*, *CACNA1H*, *SYNGAP1* and *SCN1B*.<sup>4,6-14</sup> Many of the patients presented a pre-existing intellectual disability (ID) therefore showing atypical clinical presentation<sup>15</sup> (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 <sup>12</sup>
<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 <sup>4</sup>
<i>CHD2</i>	1	41 months	Normal	Atypical: PPR Typical: generalized SW wave, slow background activity	Trivisano et al. 2015 <sup>6</sup>
<i>SCN1A</i>	1	36 months	Normal	Typical: generalized PSW	Dimova and Kietzmann 2010 <sup>16</sup>
	1	18 months	Normal	Atypical: PPR Typical: generalized SW, slow background activity	Yordanova et al. 2011 <sup>11</sup>
<i>GABRG2</i>	1	Not reported	Not reported	Not reported	Wallace et al. 2001 <sup>8</sup>
<i>CACNA1H</i>	4	Not reported	Not reported	Not reported	Heron et al. 2007 <sup>9</sup>
<i>SCN1B</i>	1	Not reported	Not reported	Not reported	Wallace et al. 1998 <sup>7</sup>
<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 <sup>14</sup>
<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 <sup>13</sup>

Abbreviations: MAE, myoclonic-atic 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),<sup>17</sup> SAMtools<sup>18</sup> 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> <sup>a</sup>	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: .959)
					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> <sup>a</sup>	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> <sup>a</sup>	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> <sup>a</sup>	MI	NM_012280.3(FTSJ1): c. <sup>a</sup> 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> <sup>a</sup>	CH	NM_000391.3(TPP1): c.509-1G>	—	Essential splicing	Essential splicing	Essential splicing
					NM_000391.3(TPP1): c.622C > T; p.Arg208 <sup>a</sup>	ExAC, ClinVar, COSMIC, ESP	Stop-gained	Stop-gained	Stop-gained
Gene not reported in neurological diseases									
11	M	N	<i>SUN1</i> <sup>a</sup>	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.

<sup>a</sup>Autism 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).<sup>33</sup>

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, <sup>19</sup> Trivisano et al. 2015 <sup>6</sup>	Carvill et al. 2013, <sup>19</sup> Trivisano et al. 2015 <sup>6</sup>	Carvill et al. 2013, <sup>19</sup> Trivisano et al. 2015 <sup>6</sup>	Trivisano et al. 2015 <sup>6</sup>	DNA recombination and cell-cycle regulation	atypical DS, LGS, Jeavons syndrome and MAE	AD	AD
<i>CACNA1H</i>	Heron et al. 2007 <sup>9</sup>	Heron et al. 2007 <sup>9</sup>	Heron et al. 2007, <sup>9</sup> Splawski et al. 2006 <sup>20</sup>	Heron et al. 2007, <sup>9</sup> Splawski et al. 2006 <sup>20</sup>	T-type calcium channel	absence epilepsy, idiopathic generalized epilepsy, febrile seizures and MAE	AD	AR
<i>KCNA2</i>	—	Syrbe et al. 2015 <sup>21</sup>	Syrbe et al. 2015 <sup>21</sup>	Syrbe et al. 2015 <sup>21</sup>	Voltage-gated potassium channel	early childhood onset EE	AD	AD
<i>STXBP1</i>	—	Saitsu et al. 2008, <sup>22</sup> Stamberger et al. 2016 <sup>23</sup>	Saitsu et al. 2008, <sup>22</sup> Stamberger et al. 2016 <sup>23</sup>	Saitsu et al. 2008, <sup>22</sup> Stamberger et al. 2016 <sup>23</sup>	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 <sup>24</sup>	Barcia et al. 2012 <sup>24</sup>	Barcia et al. 2012 <sup>24</sup>	Sodium-activated potassium channel	EIMFS and nocturnal frontal lobe epilepsy	AD	AD
<i>MECP2</i>	—	Villard 2007 <sup>25</sup>	Villard 2007 <sup>25</sup>	Villard 2007 <sup>25</sup>	Connection and synaptic regulation	Rett syndrome and severe ID	X-linked	X-linked
<i>SHROOM4</i>	—	—	Hagens et al. 2006, <sup>26</sup> Yoder and Hildebrand 2007 <sup>27</sup>	Hagens et al. 2006, <sup>26</sup> Yoder and Hildebrand 2007 <sup>27</sup>	Cytoskeletal architecture regulation	X-linked ID	X-linked	—
<i>FTSJ1</i>	—	—	Ramser et al. 2004, <sup>28</sup> Freude et al. 2004 <sup>29</sup>	Ramser et al. 2004, <sup>28</sup> Freude et al. 2004 <sup>29</sup>	Supposed translation regulation	X-linked ID	X-linked	X-linked
<i>TPP1</i>	—	—	Sun et al. 2013 <sup>30</sup>	Sun et al. 2013 <sup>30</sup>	Lysosomal serine protease	ceroid lipofuscinosis 2 and spinocerebellar ataxia	AR	AR
<i>KLC2</i>	—	—	—	Melo et al. 2015 <sup>31</sup>	Anterograde axoplasmatic transport	spastic paraplegia, optic atrophy and neuropathy	AR	AR
<i>SUN1</i>	—	—	—	Zhang et al. 2009 <sup>32</sup>	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.<sup>24,34</sup>

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 *KCL2*.

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).<sup>30,35</sup>

### 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.<sup>35</sup>

### 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*.<sup>4,12</sup> 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.<sup>6,15,19</sup> Patients present common characteristics as previous developmental delay, fever and photosensitivity. Myoclonic seizures and ASD are

often reported.<sup>6,15</sup> 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,<sup>9</sup> but also in patients with ASD and generalized epilepsy.<sup>9,20</sup> 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*.<sup>22,23,36</sup> The mutation reported in *KCNT1* was already reported in both major phenotypes, EIFMS and ADNFLE.<sup>24,34</sup> A few patients were reported with Ohtahara syndrome. Mutations in *MECP2* in males are responsible for severe ID<sup>25</sup> 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.<sup>26,28</sup> *KLC2* mutations have been reported in neurodegenerative disorder combining spastic paraplegia, optic atrophy and neuropathy.<sup>31,35</sup>

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.<sup>37,38</sup> 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

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