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## **Genetic and neurodevelopmental spectrum of SYNGAP1-associated intellectual disability and epilepsy**

Cyril Mignot,<sup>1,2,3\*</sup> Celina von Stülpnagel,<sup>4,5\*</sup> Caroline Nava,<sup>1,6\*</sup> Dorothée Ville,<sup>7</sup> Damien Sanlaville,<sup>8,9,10</sup> Gaetan Lesca,<sup>8,9,10</sup> Agnès Rastetter,<sup>6</sup> Benoit Gachet,<sup>6</sup> Yannick Marie,<sup>6</sup> G. Christoph Korenke,<sup>11</sup> Ingo Borggraefe,<sup>12</sup> Dorota Hoffmann-Zacharska,<sup>13</sup> Elżbieta Szczepanik,<sup>14</sup> Mariola Rudzka-Dybała,<sup>14</sup> Uluç Yiş,<sup>15</sup> Hande Çağlayan,<sup>16</sup> Arnaud Isapof,<sup>17</sup> Isabelle Marey,<sup>1</sup> Eleni Panagiotakaki,<sup>18</sup> Christian Korff,<sup>19</sup> Eva Rossier,<sup>20</sup> Angelika Riess,<sup>21</sup> Stefanie Beck-Woedl,<sup>21</sup> Anita Rauch,<sup>22</sup> Christiane Zweier,<sup>23</sup> Juliane Hoyer,<sup>23</sup> André Reis,<sup>23</sup> Mikhail Mironov,<sup>24</sup> Maria Bobylova,<sup>24</sup> Konstantin Mukhin,<sup>24</sup> Laura Hernandez-Hernandez,<sup>25</sup> Bridget Maher,<sup>25</sup> Sanjay Sisodiya,<sup>25</sup> Sarah Wechuysen,<sup>6,26</sup> Candace T. Myers,<sup>27</sup> Heather C. Mefford,<sup>27</sup> Konstanze Hörtnagel,<sup>28</sup> Saskia Biskup,<sup>28</sup> EuroEPINOMICS-RES MAE working group, Johannes R. Lemke,<sup>29</sup> Delphine Héron,<sup>1,2,3,4</sup> Gerhard Kluger,<sup>4,5</sup> Christel Depienne<sup>1,6</sup>

**1** AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Département de Génétique, F-75013, Paris, France

**2** Centre de Référence "Déficiences Intellectuelles de Causes Rares", F-75013, Paris, France

**3** Groupe de Recherche Clinique (GRC) "Déficience Intellectuelle et Autisme" UPMC, F-75013, Paris, France

**4** Hospital for Neuropediatrics and Neurological Rehabilitation, Epilepsy Center for Children and Adolescents, 83569 Vogtareuth, Germany

**5** Paracelsus Medical University Salzburg, Austria

- 6** Sorbonne Universités, UPMC Univ Paris 06, INSERM UMR S 1127, CNRS UMR 7225, ICM, F-75013, Paris, France
- 7** Service de Neurologie Pédiatrique, Hôpital Femme Mère Enfant, CHU de Lyon, Bron, France
- 8** Service de Génétique, Groupement Hospitalier Est, Hospices Civils de Lyon, 69677 Bron, France
- 9** Université Claude-Bernard Lyon 1, 69100 Villeurbanne, France
- 10** CRNL, CNRS UMR 5292, INSERM U1028, bâtiment IMBL, 69621 Villeurbanne, France
- 11** Klinikum Oldenburg, Zentrum für Kinder- und Jugendmedizin (Elisabeth Kinderkrankenhaus), Klinik für Neuropädiatrie u. angeborene Stoffwechselerkrankungen, D- 26133 Oldenburg, Germany
- 12** Department of Pediatric Neurology and Developmental Medicine and Epilepsy Center, University of Munich, Munich, Germany
- 13** Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland
- 14** Clinic of Neurology of Child and Adolescents; Institute of Mother and Child, Warsaw, Poland
- 15** Department of Pediatrics, Division of Child Neurology, School of Medicine, Dokuz Eylül University, İzmir, Turkey
- 16** Boğaziçi University, Department of Molecular Biology and Genetics Istanbul, Turkey
- 17** AP-HP, Hôpital Trousseau, Service de Neuropédiatrie, Paris, France
- 18** Epilepsy, Sleep and Pediatric Neurophysiology Department (ESEFNP), University Hospitals of Lyon (HCL), France

**19** Département de l'Enfant et de l'Adolescent, Neuropédiatrie - Hôpitaux

Universitaires de Genève, 1211 Genève, Switzerland

**20** g e n e t i k u m ®, Genetic Counseling and Diagnostic, Stuttgart and Neu-Ulm,

Germany

**21** Institute of Medical Genetics and Applied Genomics, University of Tübingen,

Tübingen, Germany

**22** Institute of Medical Genetics, University of Zurich, Schwerzenbach 8603,

Switzerland

**23** Institute of Human Genetics, Friedrich-Alexander-Universität Erlangen-Nürnberg,

Erlangen, Germany

**24** Svt. Luka's Institute of Child Neurology and Epilepsy, Moscow, Russia

**25** Department of Clinical and Experimental Epilepsy, Institute of Neurology,

University College London, London, and Epilepsy Society, UK

**26** Neurogenetics Group, Department of Molecular Genetics, VIB, Antwerp, Belgium

**27** Division of Genetic Medicine, Department of Pediatrics, University of Washington,

Seattle, USA

**28** CeGaT GmbH, Tübingen, Germany

**29** Institut für Humangenetik, Universitätsklinikum Leipzig, 04103 Leipzig, Germany.

\* These authors contributed equally to this work.

Correspondence to Dr. Depienne ([christel.depienne@upmc.fr](mailto:christel.depienne@upmc.fr)) or Dr. Mignot

([cyril.mignot@psl.aphp.fr](mailto:cyril.mignot@psl.aphp.fr))

## **ABSTRACT**

**Objective:** We aimed to delineate the neurodevelopmental spectrum associated with *SYNGAP1* mutations and to investigate genotype-phenotype correlations.

**Methods:** We sequenced the exome or screened the exons of *SYNGAP1* in a total of 251 patients with neurodevelopmental disorders. Molecular and clinical data from patients with *SYNGAP1* mutations from other centers were also collected, focusing on developmental aspects and the associated epileptic phenotype. A review of *SYNGAP1* mutations published in the literature was also performed.

**Results:** We describe 17 unrelated affected individuals carrying 13 different novel loss-of-function *SYNGAP1* mutations. Developmental delay was the first manifestation of *SYNGAP1*-related encephalopathy; intellectual disability became progressively obvious and was associated with autistic behaviors in half of the patients. Hypotonia and unstable gait were frequent associated neurological features. With the exception of one patient who experienced a single seizure, all patients had epilepsy, characterized by falls or head drops due to atonic or myoclonic seizures, (myoclonic) absences, and/or eyelid myoclonia. Photosensitivity was frequent. Seizures were pharmacoresistant in half of the patients. The severity of the epilepsy did not correlate with the presence of autistic features or with the severity of cognitive impairment. Mutations were distributed throughout the gene, but spared spliced 3' and 5' exons. Seizures in patients with mutations in exons 4-5 were more pharmacoresponsive than in patients with mutations in exons 8-15.

**Conclusion:** *SYNGAP1* encephalopathy is characterized by early neurodevelopmental delay typically preceding the onset of a relatively recognizable epilepsy comprising generalized seizures (absences, myoclonic jerks) and frequent photosensitivity.

## INTRODUCTION

The human *SYNGAP1* gene on chromosome 6p21.3 encodes the synaptic RAS-GTPase-activating protein 1, a protein of the post-synaptic density (PSD) of glutamatergic neurons [1, 2]. SYNGAP1 interacts with PSD95 (*DLG4*) and SAP102 (*DLG3*), and is able to positively or negatively regulate the density of NMDA and AMPA receptors at the glutamatergic synapses and mediate signaling downstream of glutamate receptor activation [3, 4]. While complete *Syngap1* deficiency in mice is lethal at early post-natal stages, heterozygous *syngap1*<sup>+/-</sup> mice are viable but show behavioral and cognitive disturbances [5, 6, 7, 8]. *Syngap1* haploinsufficiency disrupts the excitatory/inhibitory balance in the developing hippocampus and cortex and results in accelerated glutamatergic synapse maturation. When this process occurs during critical developmental windows, it alters the synaptic plasticity necessary for the refinement of connections that ultimately shape cognitive and behavioral modalities [4, 9]. Different SYNGAP1 protein isoforms exist and are generated through alternative splicing and alternative promoter usage, in a process regulated by synaptic activity and postnatal age in mice. Two of the main SYNGAP1 mouse isoforms that differ in their N-terminal and C-terminal sequences, have opposite effects on glutamate activation pathway [10]. Although several isoforms have also been described in humans, their specific role has not yet been established.

Recently, several groups have independently reported *de novo* SYNGAP1 mutations in patients with intellectual disability (ID), epileptic encephalopathy (EE) or autism spectrum disorders (ASD) identified by exome sequencing [11, 12, 13, 14, 15] or direct sequencing of the *SYNGAP1* gene through a candidate gene approach [16, 17, 18, 19, 20, 21, 22, 23, 24]. Recently, seven SYNGAP1 mutations were identified by

exome sequencing in a series of 1,133 patients, 83% of whom had ID, indicating a frequency of *SYNGAP1* mutation of ~0.74% in patients with ID [25]. One patient with a chromosomal translocation interrupting *SYNGAP1* [26] and five patients with 6p21.3 deletions encompassing *SYNGAP1* [23, 27, 28, 29, 30] have also been reported. Thus, to date, *SYNGAP1* appears one of the most relevant ID-causing genes, with mutations possibly explaining 0.7 to 1% of ID. Genotype-phenotype correlations have not been clearly established. Moreover, because most patients with *SYNGAP1* mutation were identified in large-scale exome or panel studies, the clinical features and the natural history of the *SYNGAP1*-associated ID and epilepsy remain to be precisely described. Here, we have gathered the molecular and clinical data of 15 unreported and two previously reported patients to investigate in more detail the *SYNGAP1* mutational and neurodevelopmental spectra.

## **METHODS**

**Patients.** We analyzed 251 patients with variable neurodevelopmental phenotypes including ID, EE and ASD (see Supplementary Methods for details) by exome sequencing (n=59) or direct sequencing of genes encoding synaptic proteins (n=192). One additional patient had an intragenic *SYNGAP1* deletion identified by microarray-based comparative genomic hybridization (array-CGH). Clinical and molecular data of 13 additional patients with *SYNGAP1* mutation, identified in 12 other centers, were collected: all patients with a mutation introducing a premature termination codon or occurring *de novo* (i.e. proven pathogenic), with the exception of patients with genomic deletions encompassing other genes than *SYNGAP1*, were eligible for inclusion. Patients #2 and #10 have been previously reported [12, 24]. Each patient's referring physician filled out a table with detailed developmental, neurological, behavioral and epileptic medical history, including EEG and imaging data if available. Most patients were evaluated according to developmental scales routinely used in enrolled centers by clinicians trained in neurodevelopment or neuropsychologists (for example Brunet-Lezine, HAWIK-IV, or SON-R2 scales). The sex ratio was 8 males / 9 females. Mean age at the time of the study was 10.3 years (range 3-29 years). Informed written informed consent was locally obtained for all participants. This study was approved by INSERM (RBM C12-06) and the ethical CCPRB committee from La Pitié-Salpêtrière (Paris, France).

**Exome sequencing.** The exome of index cases or parent-offspring trios was sequenced by IntegraGen (Evry, France) or by the Genotypic and sequencing facility of ICM [31]. Exons were captured from fragmented genomic DNA samples using the SureSelect Human All Exon 50Mb exome kit (Agilent Technologies) or the SeqCap



EZ Solution-Based Enrichment v3.0 (Roche), and paired-end 150-base massive parallel sequencing was carried out on an Illumina HiSeq2500 or a NextSeq500, according to manufacturers' protocols. Bioinformatics analyses were respectively done using the in-house pipeline developed by Integragen SA, as previously described [31] or by the iCONICS ICM facility platform as follows: sequencing reads passing quality filtering were aligned to the human reference genome (hg19) with Burrows-Wheeler Aligner (BWA) [32]; GATK [33] was used to recalibrate base quality scores, realign around indels, and mark duplicate reads. Variants were filtered based on their impact on the gene (missense, nonsense, frameshift, splice site-altering variants) and a minor allele frequency lower than 1% in databases (Exome Variant Server, 1000 Genomes, HapMap, Exome Aggregation Consortium, and in-house databases). Calling of *de novo* variants in trios was done using the Eris interface (Integragen SA) or Polyweb (University Paris-Descartes).

**SYNGAP1 screening and Sanger sequencing.** All exons and intron-exon junctions of *SYNGAP1* (NM\_006772.2) and 18 other synaptic genes were amplified using the Fluidigm Access Array technology (IFC Controller AX, FC1 Cyclor, 48x48 Access Arrays) and sequenced on a MiSeq Illumina sequencer as paired-end 2 x 250 bp reads. Alignment of reads on the human reference was performed with BWA and GATK, and additional bioinformatics steps including filtering for novel coding variants, were done using an in-house pipeline. Mutations identified by next generation sequencing (exome or panel) were validated by Sanger sequencing. *De novo* occurrence was tested by analyzing available parents. The predicted effect of mutations was interpreted with Alamut 2.2 (Interactive Biosoftware).

**SYNGAP1 isoforms and genotype-phenotype correlations.** Human *SYNGAP1* cDNA and protein sequences were retrieved from NCBI and Uniprot, aligned using Clustalw2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) and compared to mouse and rat isoforms [10]. We first assessed genotype-phenotype correlations in the 17 affected individuals from our cohort.

**Review of individuals with previously published *SYNGAP1* mutations.** The terms 'SYNGAP1' and 'mutation' were used to search for articles reporting patients with SYNGAP1 mutation in Pubmed. In addition, SYNGAP1 mutations and variants present in the HGMD professional (Biobase) and Exac databases were retrieved, listed and visualized on the schematic representation of the *SYNGAP1* gene. Statistical analysis was done using the Fisher exact test.

## RESULTS

**Genetic analyses and review of *SYNGAP1* mutations.** In our cohort of 251 patients with neurodevelopmental disorders, we identified 3 patients (1.2%) with novel *de novo* pathogenic heterozygous mutations of *SYNGAP1* using exome or panel sequencing. One additional patient had a *SYNGAP1* deletion of 16.6 Kb encompassing exons 2-9, identified by array-CGH. We collected additional phenotypic information for two cases published previously [12, 24] and 11 additional patients with *SYNGAP1* mutations identified in other centers (Table 1 and Supplementary Table 2).

*SYNGAP1* mutations occurred *de novo* in all 12 patients for whom DNA of both parents was available and, with the exception of one *de novo* missense mutation, all of them introduced a premature termination codon in the protein sequence (Table 1 and Figure 1). None of the mutations were reported in control databases (Exome Variant Server, 1000Genomes, HapMap, Exome Aggregation Consortium). The single missense mutation of this study (c.1685C>T, p.Pro562Leu, rs397514670), also identified in a previously reported patient [20], altered a highly conserved amino acid of the RasGap/GTPase domain of the protein (up to yeast) and was predicted damaging by SIFT and Polyphen-2.

In total, 47 patients (including two monozygotic twins [23]) carrying 43 different point mutation or indels limited to the *SYNGAP1* gene have been described to date (Figure 1 and Supplementary Table 3). Three recurrent mutations (c.321\_324del, c.427C>T/ p.Arg143\*, c.1685C>T/ p.Pro562Leu) were found in 2 patients each. Pathogenic mutations in *SYNGAP1* are distributed throughout the gene, especially in exons 5, 8, and 15, which are amongst the largest exons of *SYNGAP1*. Interestingly, the two first and two last exons, which are alternatively spliced and included in 3 out of 5

SYNGAP1 isoforms, but also exons 9 and 16, present in all known isoforms seem to be spared (Figure 1).

**Clinical and neurodevelopmental features of *SYNGAP1*-related encephalopathy (Table 1 and Supplementary Table 1).** All patients with *SYNGAP1* anomalies of our series had ID which was evaluated as severe in 10 patients, moderate in five and mild in two. The mean age of sitting unsupported was 12 months (median age 10 months, n=15) and of walking 27.7 months (median age 24 months, n=15). Half of the patients could walk by age 2 years and 75% by age 3 years. All patients had speech delay: 12 of them spoke first words at a mean age of 2.5 years and five patients did not speak at age 10 years or older. In most patients, both receptive and expressive languages were affected. Two patients had mild ID, including one without motor delay. In those, mild, progressive language delay and behavioral anomalies were the most prominent features.

Eight out of 16 patients (50%) older than 3 years old were diagnosed with ASD. Patients with ASD had remarkably poor verbal and non verbal communication abilities as well as impaired social interactions (Supplementary Table 1). Half of the patients (n=4/8) with severe ID, 1/5 with moderate ID and 2/2 with mild ID were diagnosed with ASD. Independent from a formal diagnosis of ASD, many of the patients exhibited stereotypies (n=10), temper tantrums, aggressiveness, self-injurious behavior and/or restlessness (n=7).

Neurological examination, performed at a mean age of 8.9 years, was considered normal in two patients. Gait was clumsy or unsteady in five patients and ataxic in five others. Truncal hypotonia was reported in 10 patients and facial hypotonia in four. Some patients had orthopedic problems, such as *pes planus* and rotation of the hips.

Brain MRI performed in all 17 patients (mean age 5.4 years) was either normal or revealed nonspecific features (arachnoid cysts in two patients, mild myelination delay in one, and signal abnormalities in another).

Epilepsy was diagnosed in 16/17 patients (Table 2). The only patient without epilepsy, who was aged 5 at the time of this study, had a single afebrile seizure at the age of 3.5 years. Excluding this patient, first seizures occurred at a mean age of 3 years (range: 1-8 years) and consisted of drop-attacks, massive myoclonic jerks, atonic seizures, myoclonic absences or absences. A diagnosis of Doose syndrome (DS) and epilepsy with myoclonic absences (EMA) was made in three and one patients, respectively. The others were diagnosed with unclassified genetic generalized epilepsy (GGE). None had a diagnosis of Lennox-Gastaut syndrome (LGS).

The epilepsy responded to a single anti-epileptic drug (AED), mostly sodium valproate, in seven patients and was pharmaco-resistant in nine. During the active phases of epilepsy, seizures occurred daily in five patients, 10 times per day or more in two and 100 times daily or more in two others. Seizures were of short-duration and the most frequent seizure types were typical or atypical absences (n=9), massive myoclonic jerks with or without falls (n=7), eyelid myoclonia (n=3), clonic or tonic clonic seizures (n=3), myoclonic absences (n=3) and atonic seizures (n=2). Head drops or falls were relatively frequent (n=5) and reported as myoclonic-astatic, atonic seizures or drop-attacks. Eight patients had several seizure types. No patients had status epilepticus and exacerbation by fever was mentioned in four. We found no correlations between the diagnosis of ASD and the age at epilepsy onset. The proportion of patients with ASD was identical among those with pharmaco-resistant (n=5/10) and pharmacosensitive epilepsy (n=3/6).

The most frequent anomalies reported on EEG traces (Figure 2) from 16 patients were ictal or interictal bursts of spikes, spike-waves or slow waves that were either generalized (n=13), generalized with a posterior predominance or posterior only (n=5). Paroxysmal anomalies were localized to central regions in six instances. Triggers of seizures were identified in seven patients, including photosensitivity (PS, n=5), fixation-off sensitivity (FOS, n=1), PS and FOS (n=1), and chewing (n=1).

**Genotype/phenotype correlations.** We observed no definite correlation between the location of the mutation on the gene and the severity of ID or ASD diagnosis. However, schematic representation of the clinical features of our 17 patients, ordered by the position of the mutation on the gene (Figure 3), revealed that the epilepsy of patients with mutations in exons 4-5 was mainly pharmacosensitive (5/6 patients) whereas that of patients with mutations in exons 8-15 was mainly pharmacoresistant (8/9,  $p=0.01$ ).

## **DISCUSSION**

In this study, we collected the comprehensive molecular and clinical data of the largest series of patients with *SYNGAP1* mutation so far in order to describe more accurately the neurodevelopmental and epileptic phenotype and to address genotype-phenotype correlations. Delineation of the phenotype from 36 patients with *SYNGAP1* mutations showed that it includes mild to severe ID in all, generalized epilepsy in most and autistic behavior in a half of them (Supplementary Table 3). In the present study, we describe the phenotype of 17 cases with *SYNGAP1*-associated encephalopathy, bringing the total number of reported patients with *SYNGAP1* mutations to 47.

**Neurological examination in *SYNGAP1*-associated encephalopathy.** Truncal hypotonia, sometimes in association with facial hypotonia, was the main recurrent feature in our patients, in line with previous series [20, 23]. Likewise, ataxia, with a broad-based or clumsy gait, was frequent in our patients and recurrently mentioned in others [20, 23]. Gait abnormalities are probably due to a combination of hypotonia, lack of global coordination, poor motor control, inattentiveness and orthopedic issues. Occipitofrontal circumference (OFC) was normal in 78% of patients from the literature and in 100% of ours. Though microcephaly has been mentioned in some cases [17, 20, 23], it seems to be not a common aspect in patients with *SYNGAP1* mutations. As with previously-reported patients, MRI in our patients showed either no or nonspecific features, implying that brain imaging is not helpful in the diagnosis of *SYNGAP1*-related disorders.

**The neurodevelopmental phenotype in *SYNGAP1*-associated encephalopathy.**

In our series as well as in the literature, early motor delay with severe language impairment is the first manifestation of *SYNGAP1* encephalopathy. Fourteen patients of our series acquired a few words between 1 and 4 years old but only three patients were able to speak simple sentences. These data highlight that language acquisition in most patients with *SYNGAP1* mutation rapidly reaches a plateau. It may even be subjected to regression, since seven of our patients acquired a few words but eventually lost them again during the first years of life.

Slowing of global development and seizures appeared to occur concurrently in some patients, suggesting that *SYNGAP1* mutation might be a cause of EE, as previously suggested [18]. By definition, EE is an epileptic disorder in which the "epileptic

activity itself may contribute to severe cognitive and behavioral impairments above and beyond what might be expected from the underlying pathology alone" [34]. The concept of EE may apply to specific syndromes (West syndrome and LGS) usually associated with ID or to epileptic individuals with an encephalopathic course [34]. West syndrome and LGS were not diagnosed in our patients. However, retrospective analysis of the clinical history of some of them may illustrate an "encephalopathic course" apparently related to frequent daily seizures. As an example, patient #14 in whom first seizures occurred up to 100 times a day had increasing behavioral disturbances and a concomitant stagnation of cognitive acquisition; her language and communication skills significantly improved once the epilepsy was controlled. On the contrary, the epilepsy of patient #4 responded to sodium valproate alone at 4 years old but her cognitive evolution was very poor at 10 years. Beyond these particular clinical histories, a global view of the epilepsy and neurodevelopmental disorder in our series shows that the level of ID is not related to the resistance or sensitivity of the epilepsy to AED (Figure 3). In addition, the age at first seizure does not correlate with the resistance to AED and is not clearly linked to the severity of ID. Finally, among the eight patients with language regression reported here, two of them only had a concomitant first seizure. Epilepsy in the others started several months or years after language regression. The contribution of interictal EEG abnormalities to cognitive regression is theoretically possible, but cannot be demonstrated since EEG were recorded after the first seizure. Consequently, while the concept of EE may possibly correspond to the encephalopathic course of a subgroup of patients with pharmaco-resistant epilepsy in our series, evidence to extend this concept to *SYNGAP1*-related neurodevelopmental disorder in general is lacking.



**Epilepsy in *SYNGAP1*-associated encephalopathy.** *SYNGAP1* mutation rate was 0.74% in a large series of 940 patients with ID [25], and up to 1% (5/500) in another large series of patients with EE [18]. Overall, about 85% patients with *SYNGAP1* mutations had seizures. This suggests that epilepsy is extremely common in the *SYNGAP1*-associated encephalopathy and that *SYNGAP1* is one of the most frequently mutated genes in patients with ID and epilepsy. All patients in our series had generalized seizures, like those reported in a previous study [20], only a few of them also experienced focal clonic or tonic clonic seizures. Generalized bursts of spikes, spike-waves and slow waves, sometimes with an occipital predominance, were the main recurrent EEG features in our patients. Thus, falls and myoclonic jerks, (typical or atypical) absences, sometimes in combination, define the most common seizures types that, together with the finding of interictal generalized and/or occipital anomalies on EEG, may guide toward the diagnosis of *SYNGAP1* mutation in patients with ID.

Though most of our patients with *SYNGAP1* mutations had a diagnosis of unclassified GGE, seizure types were suggestive of epilepsy syndromes associated with ID, particularly EMA and DS, which diagnosis have been suggested in 3 and 1 patient(s), respectively. To our knowledge, two other patients with EMA were found to carry a *de novo* genetic anomaly affecting *SYNGAP1*: one with a frameshift mutation [20] and another with a gene interruption due to a balanced translocation [26]. However, the sequencing of *SYNGAP1* in four other patients with EMA and in another one with DS failed to reveal any mutations. This result is in agreement with a previous work in which a single *SYNGAP1* mutation was identified in three patients with EMA, 10 with DS and two with LGS [20]. This suggests that *SYNGAP1* mutations are relatively uncommon causes of these epilepsy syndromes.

Photosensitivity has been mentioned in previously reported *SYNGAP1* patients [17, 23], but has not been emphasized. The fixation-off phenomenon has been described once [24]. In our series, photosensitivity as a trigger for seizure was found in half of the patients. Parents or caregivers of four patients noticed it as sensitivity to sunlight, artificial light or the television. This high rate of photosensitivity is significant since clinical photosensitivity is found in only 10% of patients with epilepsy in the 7-19 years old group [35]. We assume that photosensitivity may have not been detected in some of our patients because it is an age-dependent phenomenon with a peak around puberty; it could therefore still appear in some of them; or because of the poor cooperation of patients during the recording. These data suggest that photosensitivity, when present, might be a diagnostic clue from the EEG of an underlying *SYNGAP1* mutation.

**Genotype/phenotype correlations.** Although patients with *SYNGAP1* mutations show a common core clinical picture, the phenotype is relatively variable, particularly regarding the severity of ID, pharmacoresistance and the presence of ASD. Since *SYNGAP1* is a complex gene, giving rise to several protein isoforms with opposite effects on the glutamate activation pathway, via alternative splicing and transcription start sites [10], it was tempting to speculate that the location of the mutation on the gene could correlate to the clinical outcome. However, we found little correlation between the location of the mutation and the severity of ID, epilepsy and/or ASD. Yet, the epilepsy of patients with mutations in exons 4-5 appeared more pharmacosensitive than that of patients with mutations in exons 8-15. Interestingly, exons 4 and 5 are not present in *SYNGAP C*, an isoform obtained through alternative promoter usage, which existence has been demonstrated in mice and rats. Although

this isoform has not been shown to exist in humans as well, our results suggest that it could also exist and have a different function, as already proven for isoforms  $\alpha 1$  and  $\alpha 2$ , which differ in their C-terminus. Further study is necessary to confirm this finding, and decrypt the precise function of each human SYNGAP1 isoform and its relationship with the human pathology characteristics.

Nevertheless, the comparison of the clinical features of patients with identical mutations revealed significant clinical differences (Supplementary Tables 2 and 3), confirming that there is a real variability of the phenotype that depends on other factors than the mutation itself. On the contrary, monozygotic twins had strikingly similar phenotypes, suggesting that these modifier factors could be of genetic origin [23].

**ASD in SYNGAP1-associated encephalopathy and hypothetical consequences of SYNGAP1 mutations on brain development.** Although all patients with validated pathogenic SYNGAP1 mutations reported to date had ID, only half of them had a diagnosis of ASD (including data from the literature and our series). In our series, the presence of autistic traits was neither limited to patients with moderate or severe ID, nor to those with pharmaco-resistant or early-onset epilepsy. Thus, ASD, like epilepsy, could be considered as an additional feature of the SYNGAP1-related phenotype in the context of ID, irrespectively of its severity, rather than an "isolated" diagnosis.

This observation is in agreement with previous studies showing that many neurodevelopmental disorders are caused by mutations in genes encoding synaptic proteins, and more specifically constituents of the post-synaptic density [36]. The fact that a subset of patients with SYNGAP1 mutations exhibit autistic behaviors suggests

that a single mutation in a synaptic gene is not sufficient to cause ASD and that the genetic or epigenetic background of the patient probably plays an important role in the occurrence of autistic features in a context of intellectual development impairment. Many genes mutated in patients with ASD and ID are linked with neuronal signaling pathways and may alter the synaptic plasticity underlying the building, refinement and consolidation of neuronal networks associated with learning and adaptive behaviors, with the balance between inhibitory and excitatory signals being determinant in this process [37, 38, 39]. Given the function of the SYNGAP1 protein in regulating excitatory inputs downstream of NMDA receptors, the *SYNGAP1*-associated encephalopathy is likely a manifestation of the disruption of this balance. ASD as well other neurodevelopmental disorders could in many cases result from the interruption or impairment of the maturation processes of neuronal networks that are driven by neuronal activity during a critical period of brain development [39]. This scenario is particularly relevant to the fact that the clinical and morphological consequences of *SYNGAP1* haplo-insufficiency in mice, *i.e.* behavioral disturbances and premature dendrite elongation, are restricted to gene disruption during a given period of brain development [4, 9]. Following this hypothesis, *SYNGAP1* encephalopathy may be regarded as an example of premature closing of the time-window for cognitive development in humans. In the *SYNGAP1*-associated encephalopathy, disruption of the excitatory/inhibitory balance, which is also a cause of epilepsy, may therefore prematurely end the maturation process of synapses and lead to ID, ASD and epilepsy by a common pathophysiological mechanism.

## **URLS/RESOURCES**

NCBI Pubmed: <http://www.ncbi.nlm.nih.gov/pubmed>

Uniprot: <http://www.uniprot.org/>

Exome Variant Server: <http://evs.gs.washington.edu/EVS/>;

ExAC Browser (Beta) | Exome Aggregation Consortium:

<http://exac.broadinstitute.org/>

BIOBASE HGMD Professional: <http://www.biobase-international.com/product/hgmd>

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## **COMPETING INTERESTS**

The authors declare no competing interests.

## **LIST OF EUROEPINOMICS-RES MAE WORKING GROUP COINVESTIGATORS**

Dana Craiu,<sup>30,31</sup> Peter De Jonghe,<sup>26</sup> Ingo Helbig,<sup>32,33</sup> Renzo Guerrini,<sup>34</sup> Anna-Elina Lehesjoki,<sup>35,36</sup> Carla Marini,<sup>34</sup> Hiltrud Muhle,<sup>33</sup> Rikke S Møller,<sup>37</sup> Bernd Neubauer,<sup>38</sup> Deb Pal,<sup>39</sup> Kaja Selmer,<sup>40</sup> Ulrich Stephani,<sup>33</sup> Katalin Sterbova,<sup>41</sup> Pasquale Striano,<sup>42</sup> Tiina Talvik,<sup>43,44</sup> Sarah von Spiczak<sup>33</sup>

**30** Pediatric Neurology Clinic II, Department of Neurology, Pediatric Neurology, Psychiatry, Neurosurgery, “Carol Davila” University of Medicine, Bucharest, Romania

**31** Pediatric Neurology Clinic, “Professor Doctor Alexandru Obregia” Clinical Hospital, Bucharest, Romania

**32** Division of Neurology, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

**33** Department of Neuropediatrics, University Medical Center Schleswig-Holstein, Christian Albrechts University, Kiel, Germany.

**34** Pediatric Neurology Unit and Laboratories, Children's Hospital A. Meyer, University of Florence, Florence, Italy

**35** Folkhälsan Institute of Genetics, Helsinki, Finland

**36** Research Programs Unit, Molecular Neurology and Neuroscience Center, University of Helsinki, Helsinki, Finland.

**37** Danish Epilepsy Centre, Dianalund, Denmark

**38** Department of Neuropediatrics, University Medical Faculty Giessen and Marburg, Giessen, Germany

**39** Department of Clinical Neuroscience, Institute of Psychiatry, King's College London, London, UK

**40** Department of Medical Genetics, Oslo University Hospital, Oslo, Norway

**41** Child Neurology Department, University Hospital Motol, Prague, Czech Republic

**42** Pediatric Neurology and Muscular Diseases Unit, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, 'G Gaslini Institute', Genova, Italy

**43** Department of Pediatrics, University of Tartu, Tartu, Estonia.

**44** Department of Neurology and Neurorehabilitation, Children's Clinic, Tartu University Hospital, Tartu, Estonia.

### CONTRIBUTORSHIP STATEMENT.

Author	Affiliation	Design and coordination of the study	Contributing genetic and/or phenotypic data	Writing of the manuscript	Revision of the manuscript
Cyril Mignot	AP-HP, Hôpital de la Pitié-Salpêtrière, Département de Génétique, F-75013, Paris, France.	✓	✓	✓	✓
Celina von Stülpnagel	Hospital for Neuropediatrics and Neurological Rehabilitation, Epilepsy Center for Children and Adolescents, 83569 Vogtareuth, Germany.	✓	✓	✓	✓
Caroline Nava	AP-HP, Hôpital de la Pitié-Salpêtrière, Département de Génétique, F-75013, Paris, France.	✓	✓	✓	✓
Dorothee Ville	Service de neurologie pédiatrique, Hôpital Femme Mère Enfant, CHU de Lyon, Bron, France.		✓		
Damien Sanlaville	Service de génétique, groupement hospitalier Est, hospices civils de Lyon, 69677 Bron, France.		✓		
Gaetan Lesca	Service de génétique, groupement hospitalier Est, hospices civils de Lyon, 69677 Bron, France.		✓		✓
Agnès Rastetter	Sorbonne Universités, UPMC Univ Paris 06, INSERM UMR S 1127, CNRS UMR 7225, ICM, F-75013, Paris, France.		✓		
Benoit Gachet	Sorbonne Universités, UPMC Univ Paris 06, INSERM UMR S 1127, CNRS UMR 7225, ICM, F-75013, Paris, France.		✓		

Yannick Marie	Sorbonne Universités, UPMC Univ Paris 06, INSERM UMR S 1127, CNRS UMR 7225, ICM, F-75013, Paris, France.		✓		
Christoph Korenke	Klinikum Oldenburg, Zentrum für Kinder- und Jugendmedizin (Elisabeth Kinderkrankenhaus), Klinik für Neuropädiatrie u. angeborene Stoffwechselerkrankungen, D- 26133 Oldenburg, Germany.		✓		
Ingo Borggraefe	Department of Pediatric Neurology and Developmental Medicine and Epilepsy Center, University of Munich, Munich, Germany.		✓		
Dorota Hoffmann-Zacharska	Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland.		✓		
Elżbieta Szczepanik	Clinic of Neurology of Child and Adolescents; Institute of Mother and Child, Warsaw, Poland		✓		
Mariola Rudzka-Dybała	Clinic of Neurology of Child and Adolescents; Institute of Mother and Child, Warsaw, Poland.		✓		
Uluç Yiş	Department of Pediatrics, Division of Child Neurology, School of Medicine, Dokuz Eylül University, İzmir, Turkey.		✓		
Hande Çağlayan	Boğaziçi University, Department of Molecular Biology and Genetics Istanbul, Turkey.		✓		
Arnaud Isapof	AP-HP, Hôpital Trousseau, Service de Neuropédiatrie, Paris, France.		✓		
Isabelle Marey	AP-HP, Hôpital de la Pitié-Salpêtrière, Département de Génétique, F-75013, Paris, France.		✓		
Eleni Panagiotakaki	Epilepsy, Sleep and Pediatric Neurophysiology Department (ESEFNP), University Hospitals of Lyon (HCL), France.		✓		
Christian Korff	Département de l'enfant et de l'adolescent, Neuropédiatrie - Hôpitaux Universitaires de Genève, 1211 Genève, Switzerland.		✓		
Eva Rossier	Institute of Human Genetics, University of Tuebingen, Tuebingen 72076, Germany.		✓		
Angelika Riess	Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany.		✓		
Stefanie Beck-Woedl	Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany.		✓		
Anita Rauch	Institute of Medical Genetics, University of Zurich, Schwerzenbach 8603, Switzerland.		✓		
Christiane Zweier	Institute of Human Genetics, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany.		✓		✓
Juliane Hoyer	Institute of Human Genetics, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany.		✓		



André Reis	Institute of Human Genetics, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany.		✓		
Mikhail Mironov	Svt. Luka's Institute of Child Neurology and Epilepsy, Moscow, Russia.		✓		
Maria Bobylova	Svt. Luka's Institute of Child Neurology and Epilepsy, Moscow, Russia.		✓		
Konstantin Mukhin	Svt. Luka's Institute of Child Neurology and Epilepsy, Moscow, Russia.		✓		
Laura Hernandez-Hernandez	Department of Clinical and Experimental Epilepsy, Institute of Neurology, University College London, London, and Epilepsy Society, UK.		✓		
Bridget Maher	Department of Clinical and Experimental Epilepsy, Institute of Neurology, University College London, London, and Epilepsy Society, UK.		✓		
Sanjay Sisodiya	Department of Clinical and Experimental Epilepsy, Institute of Neurology, University College London, London, and Epilepsy Society, UK.		✓		✓
Sarah Weckhuysen	Neurogenetics group, Department of Molecular Genetics, VIB, Antwerp, Belgium.		✓		✓
Candace T Myers	Division of Genetic Medicine, Department of Pediatrics, University of Washington, Seattle, WA 98195, USA.		✓		
Heather C Mefford	Division of Genetic Medicine, Department of Pediatrics, University of Washington, Seattle, WA, USA.		✓		
Konstanze Hörtnagel	CeGaT GmbH, Tübingen, Germany		✓		
Saskia Biskup	CeGaT GmbH, Tübingen, Germany		✓		
Johannes Lemke	Department of Women and Child Health, Hospital for Children and Adolescents, University of Leipzig, Leipzig, Germany.		✓		
Delphine Héron	AP-HP, Hôpital de la Pitié-Salpêtrière, Département de Génétique, F-75013, Paris, France.		✓		
Gerhard Kluger	Hospital for Neuropediatrics and Neurological Rehabilitation, Epilepsy Center for Children and Adolescents, 83569 Vogtareuth, Germany.	✓	✓	✓	✓
Christel Depienne	Sorbonne universités, UPMC université Paris 06, 91-105, boulevard de l'Hôpital, 75013 Paris, France.  ICM, CNRS UMR 7225, Inserm U 1127, 47/83, boulevard de l'Hôpital, 75013 Paris, France.  Département de génétique, AP-HP, hôpital Pitié-Salpêtrière, 47/83, boulevard de l'Hôpital, 75013 Paris, France.	✓	✓	✓	✓

## FIGURE LEGENDS

**Figure 1.** Summary of *SYNGAP1* mutations identified in this study and the literature.

(A) Location of mutations on the different *SYNGAP1* isoforms. Mutations in red correspond to the patients identified in this study. Mutations in black correspond to previously published patients. Recurrent mutations are underlined. Isoform 1 corresponds to the longest isoform (NM\_006772.2, N-terminus: SYNGAP A, C-terminus: SYNGAP  $\alpha$ 2); isoform 2 is obtained through alternative splicing of exons 18 and 19 and differs in its C-terminus (SYNGAP  $\beta$ : 1265-1343: RLMLVEEELR...NGEFRNTADH  $\rightarrow$  SPSLQADAGGGGAAPGPPRHG); isoform 3 is obtained through alternative transcription start site usage involving an additional exon and differs in its N-terminus (SYNGAP B: 1-98: MSRSRASIH...PVEGRPHGEH  $\rightarrow$  MGLRPPTPSP...RRCSSCCFPG); isoform 4 is obtained through alternative splicing of exon 19 and differs in its C-terminus (SYNGAP  $\gamma$ : 1296-1343: ERQLPPLGPTNPRV...LQITENGEFRNTADH  $\rightarrow$  LLIR). Isoform 5 corresponds to a rat isoform obtained through transcription start site usage (SYNGAP C); its existence in humans has not been demonstrated and therefore remains putative. Note that other isoforms, not represented on this schematic, have been described in rodents but not yet in humans, in particular isoform alpha 1, which differs in the C-terminus (QTRV). (B) Schematic representation of the mutations (above) and the variants present in the Exome Aggregation (ExAc) database (below) on the longest *SYNGAP1* isoform (NM\_006772.2) and corresponding protein domains.

**Figure 2.** EEG samples from patients exemplifying electroencephalographic findings in *SYNGAP1*-related encephalopathy. (A) Sample demonstrating normalization of

paroxysmal activity by eye opening, i.e. fixation-off sensitivity, in Patient #2. (B) Sample showing paroxysmal activity under photic stimulation, i.e. photosensitivity, in Patient #2. (C) Sample from Patient #1: burst of generalized spikes concomitant of a rapid eye deviation (fast rhythms are due to benzodiazepine therapy). (D) Sample from Patient #12 showing the appearance of generalized spike-wave complexes with a low degree of bilateral synchronization after eye closure (fixation off phenomenon).

**Figure 3.** Graphical representation of clinical data (age at epilepsy onset, level of ID and pharmacoresistance or pharmacosensitivity) in our patients series. X-axis indicates the number of the patient, ordered by the position of the mutation on the gene, except patient 1, who corresponds to the patient with the intragenic *SYNGAP1* deletion. Y-axis indicates the age at seizure onset (in months). The proportion of patients with mild (circles), moderate (triangles) and severe (squares) ID is not different in the pharmacoresistant (red) and in the pharmacosensitive (green) groups. One patient (black square, patient 10), who had a single afebrile seizure and was thus not considered strictly as epileptic, was not considered for this analysis. The age at the first seizure is neither related to the resistance or sensitivity of the epilepsy to AED, nor to the position on the gene. The age at seizure onset is not correlated with the level of ID. The mutations of most patients with pharmacosensitive epilepsy cluster in exons 4-5 whereas those of most patients with pharmacoresistant epilepsy spread over exons 8-15 ( $p=0.001$ ).

## **SUPPLEMENTARY DATA**

**Supplementary Table 1.** Additional data to Table 1.

**Supplementary Table 2.** Molecular data of patients with *SYNGAP1*-associated encephalopathy reported in the literature and in the present study. Lines with recurrent mutations are highlighted in green.

**Supplementary Table 3.** Clinical data of patients with *SYNGAP1*-associated encephalopathy from the literature. Patients reported in two articles [21,30] were not included because of insufficient clinical data.

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Table 1. Molecular and clinical data from the 17 patients with *SYNGAP1* mutations\*. (1)

Patient ID	1	2	3	4	5	6	7	8	9	
Age at the time of the study (years)	14	15	8.5	10.8	15	11	5	9.8	5.5	
Sex	M	F	F	M	F	M	F	F	F	
Ancestry	Guinean	European	European	European	Moroccan	Malian	European	European	European	
Genetics	Mutation type	intragenic deletion	nonsense	nonsense	nonsense	frameshift	nonsense	splice site	frameshift	frameshift
	Mutation	c.68-1518-?_1530+?del	c.348C>A	c.403C>T	c.427C>T	c.455_459del	c.490C>T	c.509+1 G>T	c.828dup	c.1057delC
	Protein level	p.?	p.Tyr116*	p.Arg135*	p.Arg143*	p.Arg152Glnfs*14	p.Arg164*	p.?	p.Lys277Glnfs*7	p.Leu353Trpfs*13
	Location in gene	intron 1 - exon 9	exon 4	exon 5	exon 5	exon 5	exon 5	intron 5	exon 8	exon 8
	Inheritance	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>	parents not tested	parents not tested
Level of intellectual disability / Age at evaluation	severe / 10 y	mild / 12 y	moderate / 5.5 y	severe / 10.8 y	severe / 11 y	severe / 11 y	moderate / 5 y	moderate / 4.5 y	moderate / 5.5 y	
Developmental stages	Age of sitting / walking	7 m / 24 m	10 m / < 18 m	10 m / 20 m	10 m / 24 m	16 m / 36 m	8 m / 20 m	10 m / 22 m	9 m / 15 m	NA / 24 m
	Age of first words / first sentences	4 y / no sentences	14 m / NA	33 m / no sentences	5 y (5 words) / no sentences	4 y transient "mama" "papa" / no sentences	NA / no sentences	3 y / 5 y	23 m	36 m / no sentences
	Current language ability	single words	NA	~ 50 words	10 words	absence of speech	few words at 11 y	5-word sentences	short sentences	15 words
	Regressive episode during the development / Age	slowing of development with untreated epilepsy / 2y	no	no	no	possible (loss of few acquired words)	no	loss of few dissyllable words after 20 m	no	NA
Autism spectrum disorder	no	yes	no	yes	yes	yes	no	no	no	
Clinical Examination	Age at examination	14 y	12 y	5.5 y	10.8 y	11 y	10 y	5 y	6 y	5.5 y
	Height in cm (SD) / weight in kg (SD) / head circumference in cm (SD)	133 (-0.5) / 28 (-0.5) / 50.5 (-1)	173 (+2.5) / 40 (-1) / 53 (-1)	151 (+1) / 53 (+3) / 53.5 (-0.5)	NA	156 (-0.75) / 62 (+0.25) / NA	143 (+4) / 35 (+3.5) / 51 (-0.5)	15 (-1.5) / 103 (-1.5) / 49 (-1.5)	105 (-0.5) / 16 (-1) / 52 (+0.5)	110 (-1.5) / 17.9 (-1.5) / 50.5 (-0.5)
	Neurologic examination	normal	normal	global hypotonia, gait ataxia	truncal hypotonia	nystagmus during the 1st year (possibly caused by myopia), clumsy gait	facial and truncal hypotonia, broad based gait	truncal hypotonia	facial hypotonia with drooling, gait ataxia	truncal hypotonia, walking with inwards rotation of hips

Table 1. Molecular and clinical data from the 17 patients with *SYNGAP1* mutations\*. (2)

Patient ID	10	11	12	13	14	15	16	17	Summary	
Age at the time of the study (years)	5	3	22	12	8	8.2	29	10	mean 11.4	
Sex	M	M	F	M	F	M	M	M	M=8, F=9	
Ancestry	European	Iraqi	European	Turkish	European	European	European	European		
Genetics	Mutation type	nonsense	nonsense	missense	nonsense	frameshift	frameshift	frameshift	splice site	nonsense 7; frameshift 5; splice 2; missense 1; intragenic deletion 1
	Mutation	c.1253_1254del	c.1630C>T	c.1685C>T	c.1995T>A	c.2214_2217del	c.2933del	c.3406dup	c.3408+1G>A	
	Protein level	p.Lys418Argfs*54	p.Arg544*	p.Pro562Leu	p.Tyr665*	p.Glu739Glyfs*20	p.Pro978Hisfs*99	p.Gln1136Profs*17	p.?	
	Location in gene	exon 8	exon 10	exon 11	exon 12	exon 13	exon 15	exon 15	intron 15	
	Inheritance	<i>de novo</i>	<i>de novo</i>	<i>de novo</i>	parents not tested	<i>de novo</i>	<i>de novo</i>	parents not tested	<i>de novo</i>	
Level of intellectual disability / Age at evaluation	severe / 4 y	severe / 3 y	severe / 22 y	severe / 12 y	mild / 8 y	moderate / 5 y	severe / 8.5 y	severe / 10 y	mild n=2; moderate n=5; severe n=10 / mean age eval. 8.7 y	
Developmental stages	Age of sitting / walking	15-18 m / 36 m	12 m / walks only with aid	12 m / 38 m	NA / 36 m	8 m / 18 m	10 m / 18 m	16 m / 30 m	25 m / 4.5 y	mean 12 m / 27.7 m
	Age of first words / first sentences	~29 m transient "mama", "papa" / no sentences	3 y "papa" only / no sentences	no words / no sentences	no words / no sentences	12 m / 6 y	3 y / no sentences	17 m / no sentences	no words / no sentences	mean age first words 2.6 y
	Current language ability	absence of speech	absence of speech	absence of speech	absence of speech	120 words, 3 to 4-word sentences	5 words	absence of speech	absence of speech	absence of speech 7; speaks words 5; associates words or simple sentences 3
	Regressive episode during the development / Age	since age of 36 months-loss of "mama", "papa"	no	12m - with febrile seizures	no	14 months	no	loss of words at age 18-30 m	possible (loss of 2-syllable words)	n=7
Autism spectrum disorder	yes	too young to be evaluated	no	no	yes	no	yes	yes	yes 8; no 8	
Clinical Examination	Age at examination	5.2 y	3 y	22 y	12 y	8 y	7 y	8.5 y	6.6 y	mean 8.9 y
	Height in cm (SD) / weight in kg (SD) / head circumference in cm (SD)	149 (+1.5) / 48.6 (+2) / 52 (-1.5)	105 (-0.5) / 20 (+1.5) / 49.3 (-1)	93 (0) / 13.8 (0) / 48 (-2)	146.5 (+1) / 35 (+0.5) / 55 (+1)	NA / 21 (-1) / 54 (+1)	116 (+1) / 21 (+1) / 50 (0)	124 cm (-1.5) / 22 kg (-1.8) / 50.8 cm (-1.7)	116 cm (+0.4) / 22.3 kg (+0.7) / 51.3 cm (+0.4)	normal OFC 15/15
	Neurologic examination	truncal hypotonia, broad based gait, hypotonic-atactic movements	truncal hypotonia, swallowing difficulties	mild gait ataxia, flexion deformity of left hip, hyperlordotic lumbar spine	hyperactive deep tendon reflexes, unsteady gait	motor slowness and moderate akinesia, ataxic gait, truncal hypotonia, dystonic postures of hands and feet, plastic hypertonia	truncal hypotonia, orthostatic truncal tremor, slight pyramidal tetraparesis, gait ataxia	truncal hypotonia	truncal hypotonia, orofacial hypotonia, wide-based gait	clumsy/ataxic gait 10, truncal hypotonia 10, facial hypotonia 4, normal exam 2

\* patients are ordered by mutation from the 5' end of the gene. NA: not available; m: months; y: years; mean age eval.: mean age at evaluation; SD: standard deviation.

Table 2. Epilepsy features in SYNGAP1-related encephalopathy. (1)

Patient ID	1	2	3	4	5	6	7	8	9	
<b>Age at seizure onset (m:months or y:years)</b>	24 m	24 m	22 m	4 y	3 y	30 m	5 y	33 m	30 m	
<b>Seizure type at onset</b>	myoclonic jerks (falls)	drop attacks	febrile seizure	GTCS, abs.	tonic febrile and afebrile, myoclonic jerks	not defined	abs.	abs.	head nodding, abs.	
<b>Seizure types during disease course</b>	myoclonic abs., eye myoclonia	GTCS, clonic, drop attacks, myoclonic jerks,	atypical abs., myoclonic jerks, atonic seizures	abs.	head falls, massive myoclonic jerks of arms, myoclonic abs.	abs.	abs.	abs.	myoclonic jerks (mainly arms)	
<b>Epilepsy syndrome</b>	EMA	DS then atypical GGE	unclassified GGE	unclassified GGE with absences	unclassified GGE	unclassified GGE with absences	unclassified GGE with absences	unclassified GGE with absences	unclassified GGE with absences	
<b>Febrile seizures</b>	no	yes	yes	no	rare	no	no	no	no	
<b>Status epilepticus</b>	no	no	no	no	no	no	no	no	no	
<b>Frequency of seizures</b>	>10 daily then 2/day presently nearly seizure-free	daily -> one per week-> almost seizure free	1-2/month	seizure free for several years	controlled	<1/day	several/day	daily	up to 100/day	
<b>Lifetime / current anti-epileptic treatment</b>	VPA	VPA then LEV	LEV	VPA	VPA, OXC, LTG, LEV, CBZ / VPA + LTG	VPA, CBZ	LTG	VPA, LTG / LTG	VPA, ETH, LEV, CLN*, ketogenic diet / none	
<b>Pharmoresistance</b>	no	no	no	no	partial	no	no	yes	yes	
<b>EEG</b>	Age at examination	9 y	2 to 15 y	4.5 y	9 y	1 to 5 y	3 to 8 y	5 y	8.5 y	5 y
	Main abnormalities	generalized bursts of S	generalized PsW and photoconvulsions	frontal and generalized SpW and PSW	irregular spike-slow-wave-complexes: generalized, maximum frontal; beta-waves	1 y: normal; 3.5 y: generalized bursts of S, S + SW in posterior areas; 5 y: slow background activity, fronto-temporal bursts of SW	bi-occipital SW, S and SpW, bi-central anomalies	NA	diffuse SpW, PSp or PSW	bursts of bilateral S and PSp with maximum in posterior regions
	Triggers of seizures	none	PS	no	none	none	none	NA	none	chewing, emotions



Table 2. Epilepsy features in SYNGAP1-related encephalopathy. (2)

Patient ID	10	11	12	13	14	15	16	17	Summary
Age at seizure onset (m:months or y:years)	one seizure at 3.5 y	24 m	12 m	<2 y	5 y	22 m	27 m	8 y	mean 35.4 m, median age 28.5 m, 75th centile 39 m
Seizure type at onset	non febrile	febrile seizure	febrile seizures	astatic seizures	eyelid myoclonia	atonic	myoclonic seizures	NA	
Seizure types during disease course	NA	eyelid myoclonia	eyelid myoclonia, atypical abs., myoclonic jerks	myoclonic astatic	eyelid myoclonia, myoclonic abs.	GTCS, focal, atypical abs., myoclonic jerks	myoclonic jerks, GTCS, atypical abs.	atypical absences	myoclonic jerks 7, atypical abs. 5, abs. 4, eyelid myoclonia 3, clonic or GTCS 3, myoclonic abs.3, atonic 2
Epilepsy syndrome	NA	unclassified GGE	unclassified GGE	DS	unclassified GGE	DS	unclassified GGE	unclassified	unclassified 12, DS 3, EMA 1
Febrile seizures	no	yes	yes	no	no	no	no	no	yes 4
Status epilepticus	no	no	no	no	no	clusters of seizures/no status epilepticus	no	no	n=0
Frequency of seizures	only one until now	several/day	several/month	10/day	100/day	several/day	several/day	4-8/month	
Lifetime / current anti-epileptic treatment	no	VPA	LEV, TPM	VPA, ZNM, LTG	LEV, ETH	VPA, LTG + VPA, LTG, LEV, CLN, ACTH	VPA, CBL, TPM / ketogenic diet	VPA	
Pharmoresistance	not applicable	no	yes	yes	yes	yes	yes	partial	yes 9, no 7
EEG	Age at examination	1.8 and 2.5 y	3 y	3 to 8 y	2 to 10 y	2 to 5 y	7	8.5 y	2.3 y
	Main abnormalities	1st: SW; 2nd: no abnormalities	abnormal background, generalized slowing, recorded seizures with eyelid myoclonia and generalized seizure patterns	bursts of S and SW in the occipital region after eye closure	generalized SpW	2y: normal; 5y: ictal bursts of diffuse PSW with posterior predominance after eyes closer and photic stimulation	focal SpW in central-parietal areas, generalized S and PSW	generalized PSW and frontal Sw	multifocal SW
	Triggers of seizures	none	PS	FOS	PS	PS, FOS	none	none	PS

GTCS: generalized tonic-clonic seizures; abs.: absences; EMA: epilepsy with myoclonic absences; GGE: genetic generalized epilepsy; DS: Doose syndrome. Anti-epileptic drugs: VPA: valproic acid, LEV: levetiracetam, ETH: ethosuximide, OXC: oxcarbazepine, CBZ: clobazam, ZNM: zonisamide, LTG: lamotrigin, TPM: topiramate, CLN: clonazepam, ACTH: adrenocorticotrophic hormone. EEG: electroencephalogram; SW: slow waves; S: spikes; SpW: spike-waves; PSW: polyspike-waves; PSp: polyspikes; PS: photosensitivity; FOS: fixation off sensitivity.  
\*epilepsy aggravated

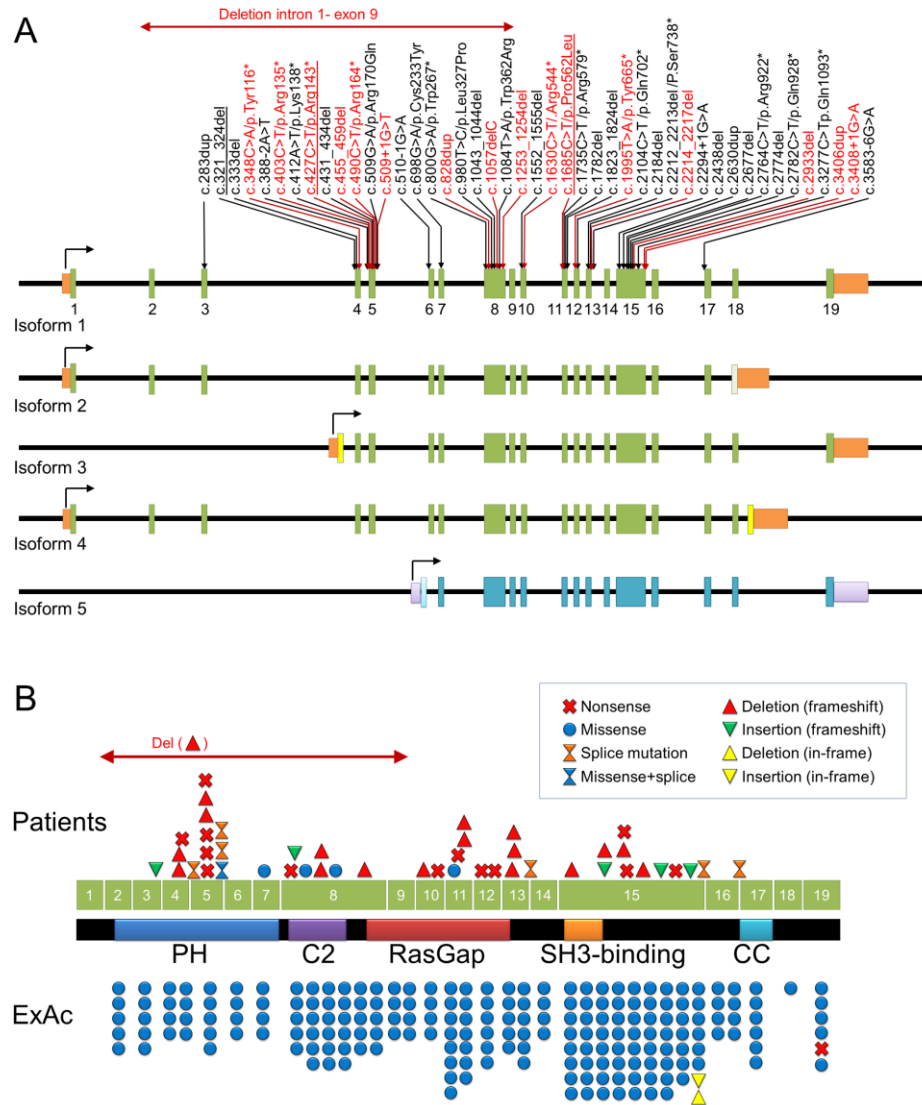


Figure 1.

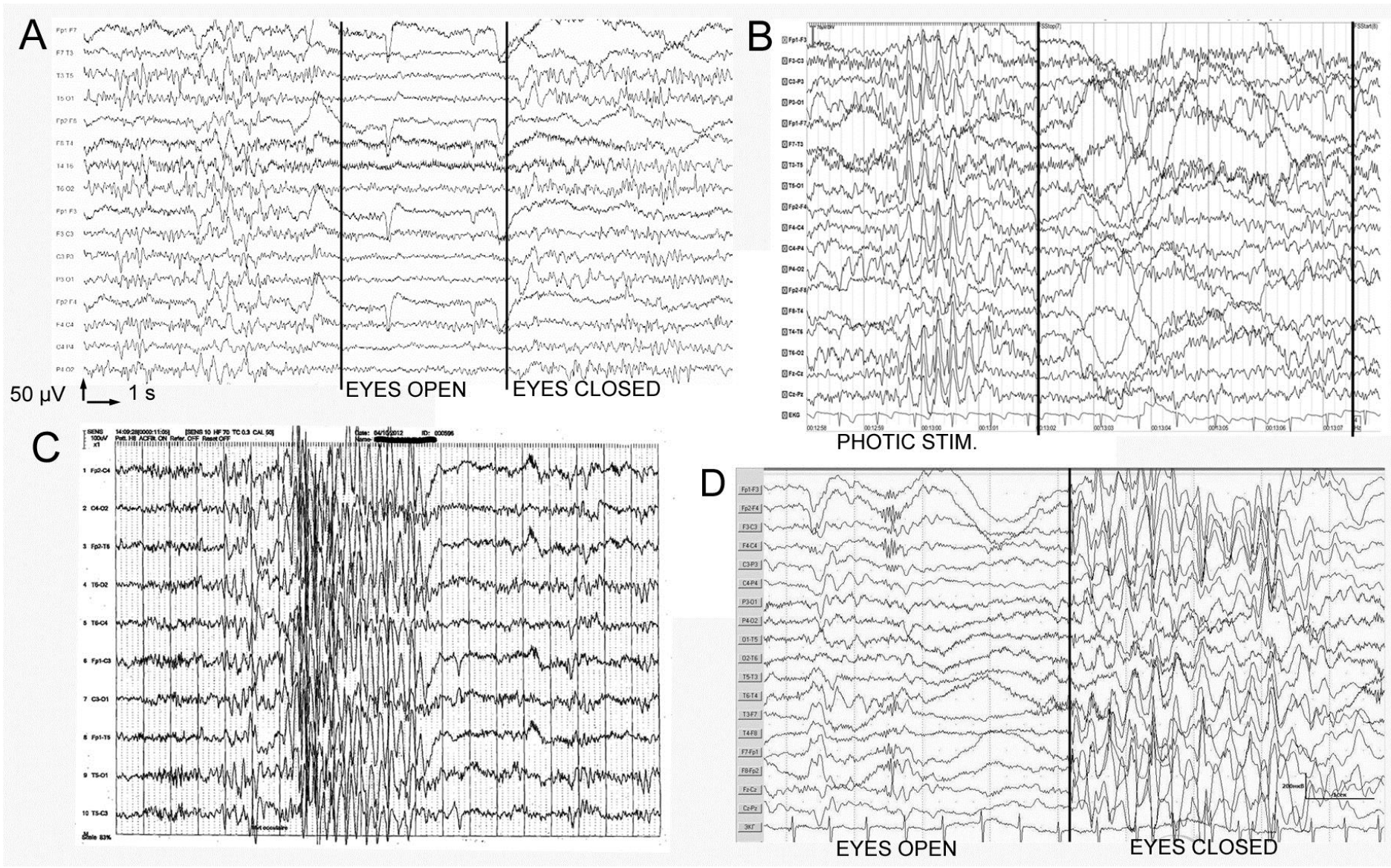


Figure 2

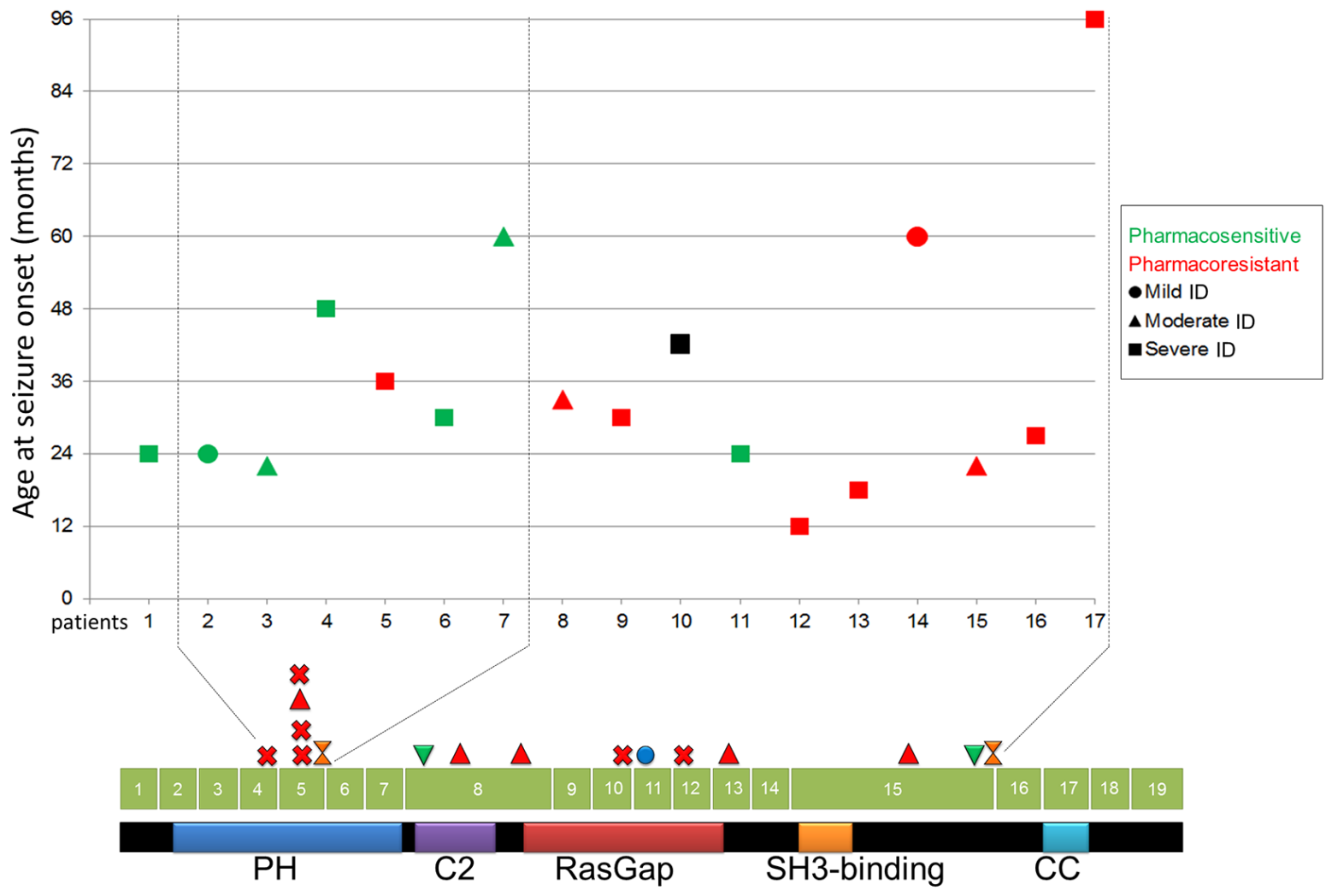
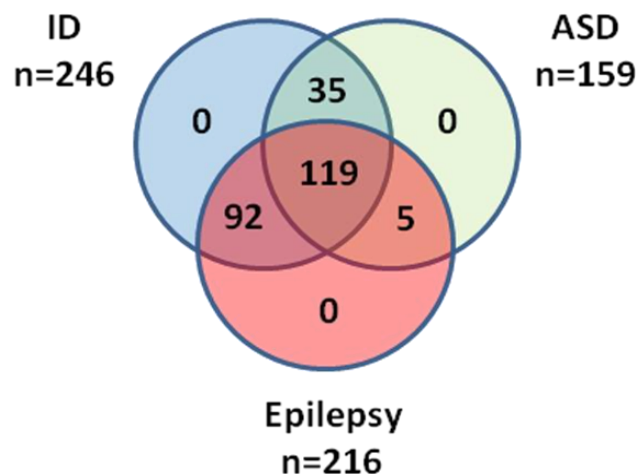


Figure 3.

### Supplementary Methods.

Clinical characteristics of the 251 patients with variable neurodevelopmental phenotypes included in this study (ID: intellectual disability, ASD: autism spectrum disorder).



Among epileptic patients, 158 had a non-syndromic or unclassified epilepsy. The epilepsy type or the main seizure type in the 58 other patients were the following: West syndrome (n=24), epilepsy with myoclonic absences (n=5), Doose syndrome/ epilepsy with myoclonic atonic seizures (n=1), malignant migrating partial seizures of infancy (n=1), unspecified neonatal epileptic encephalopathy (n=7), myoclonic epilepsy (n=4), absence epilepsy (n=3), generalized epilepsy with tonic-clonic seizures (n=13).

Supplementary Table 1. Complement to genetic and clinical data of the 17 patients with *SYNGAP1* mutations. (1)

Patient ID		1	2	3	4	5	6	7	8	9
Genetics	Family history	none	cousin with absence epilepsy	none	none	none	none	none	none	none
	Parental age at birth	Mo=35, Fa=18	NA	Mo=34, Fa=35	Mo=40, Fa=36	Mo=40, Fa=47	NA	Mo=40, Fa=30	Mo=28, Fa=28	NA
	Other significant genetics abnormalities	none	none	array-CGH: Xp22.33 dup 491 kb - 511 kb (inherited from healthy father)	<i>de novo</i> VOUS: <i>RANBP2</i> : c.8146G>A (p.K2716E); <i>KLHL8</i> : c.95C>G (p.S32L)	variant in <i>MBD5</i> inherited from one parent	none	none	none	RBFOX1-deletion (hq19 chr16:6340454-6814185)
	Method of molecular diagnosis	microarray analysis	CeGat panel	CeGat panel	WES	SYNGAP1 testing	WES	panel (genetikum®)	SYNGAP1 testing	CeGat panel
Neonatal period	Pregnancy and delivery	probably normal, fullterm	unremarkable	Apgar 8/10/10	unremarkable, cesarean section week 39, Apgar scores 10/10	unremarkable	twin pregnancy, born at 8 months, delivery unremarkable	unremarkable	mild gestational diabetes, fullterm, Apgar 10	unremarkable
	Birth length in cm (perc) / weight in g (perc) / head circumference in cm (perc)	NA	52 (50th) / 3010 (50th) / 34 (25th)	55 (90th) / 4125 (96th) / 36.5 (98th)	49 (10-50th) / 3160 (10-50th) / 34 (10-50th)	50 (10-50th) / 3230 (10-50th) / 34.5 (10-50th)	NA / 2740 (NA) / 34 (NA)	52 / 3880 / 36	48 (10th) / 3300 (25th) / 33 (25th)	48 (10th) / 3420 (10-50th) / NA
	Neonatal findings	NA	none	none	muscular hypertonia during first months	none	none	none	none	none
Autism spectrum disorder	Alteration of nonverbal communication	mild	mild	moderate	no	moderate	severe	NA	mild	severe
	Repetitive behaviours	no	no	no	yes	yes	yes	little	no	no
	Stereotypies	no	no	no	yes	yes	yes	no	yes	no
	Social interactions	normal	no social reactions to peers, lack of eye-contact, no empathy	altered	normal	very poor	very poor	altered	altered	altered
	Behaviour troubles	temper tantrum then peaceful behaviour	aggr. especially after acoustic or tactile stimuli	severe social anxiety	hetero- / autoagr.	anxiety	restlessness, aggr.	autoaggr., temper tantrum	temper tantrum, occasional aggr.	anxiety, uncontrolled panic attacks
Brain imaging (age)	normal (9 y)	normal (13 y)	normal (3.8 y)	myelination not yet complete (4 y)	normal (2 and 9 y)	normal with small cyst in the right pontocerebellar angle	normal (2 y)	normal (4 y)	normal (3 y)	

Supplementary Table 1. Complement to genetic and clinical data of the 17 patients with *SYNGAP1* mutations. (2)

Patient ID	10	11	12	13	14	15	16	17	
<b>Genetics</b>	Family history	none	none	none	none	none	none	none	
	Parental age at birth	Mo=34, Fa=35	Mo=46, Fa=38	NA	Mo=30, Fa=33	Mo=26, Fa=26	Mo=31, Fa=27	Mo=37, Fa=40	Mo=31, Fa=28
	Other significant genetics abnormalities	none	VOUS inherited from the mother: <i>SCN9A</i> : c.4282G>A and c.5624G>A, <i>ARX</i> c.1462A>G	none	none	none	none	array-CGH: 3q12.2-12.3 dup 1.55-1.60 MB (inherited from healthy father)	none
	Method of molecular diagnosis	WES	CeGaT panel	WES	WES	WES	MIP gene panel	CeGaT panel	MR-Panel (Kingsmore Panel)
<b>Neonatal period</b>	Pregnancy and delivery	unremarkable	pathologic, otherwise normal delivery, Apgar 10/10	IVF; emergency LCSC at 36 weeks due to reduced fetal movements	unremarkable	unremarkable	born at 37 WG, premature detachment of the placenta, Apgar 10	gestation diabetes, Apgar 9/10/10	36+6 week of pregnancy, APGAR 8/8/8, intensive care
	Birth length in cm (perc) / weight in g (perc) / head circumference in cm (perc)	49 (10th) / 3160 (25th) / 36 (50th)	NA	47 (50th) / NA / NA	50 (50th) / 3500 (50th) / 34 (10-50th)	49 (10-50th) / 3120 (10-50th) / 34 (10-50th)	52 (90-97th) / 2700 (25th) / 33 (10th)	49 (14th) / 3350 (46th) / 35 (52th)	47.5 (<10th) / 2490 (50th) / 33.5 (>50th)
	Neonatal findings	none	none	slow to suck and feed	none	none	hyperbilirubinemia	none	hypotonia, bradycardia, hypothermia, hyperbilirubinemia
	<b>Autism spectrum disorder</b>	Alteration of nonverbal communication	moderate-severe	moderate-severe	moderate	moderate	NA	NA	severe
Repetitive behaviours		no	yes	no	no	NA	NA	yes	yes
Stereotypies		yes	yes	yes	no	yes	yes	yes	yes
Social interactions		very poor	very poor	altered	altered	NA	NA	very poor	poor
Behaviour troubles		hetero- / autoaggr.	NA	NA	aggr.	NA	NA	auto- and hetero-aggr.	altered
<b>Brain imaging (age)</b>	arachnoid cysts (1.8 y)	normal (3 y)	normal (8 y)	normal (8 y)	normal (5 y)	slight dilatation of the anterior horns of lateral ventricles (3 and 5 y)	normal (28 m and 6 y)	bilateral T2 hypersignal of fasciculus longitudinalis medialis, pirirtrigonal white matter and central parts of centrum semiovale (15 m)	

Mo: mother; Fa: father; VOUS: variant of unknown signification; WES: whole exome sequencing; ID: intellectual deficiency; NA: not available; m: months; y: years; aggr.: aggressiveness; SD: standard deviation; WG: weeks of gestation; IVF: in vitro fertilization; LCSC: lower segment Caesarean section.

Supplementary Table 2. Molecular data of patients with *SYNGAP1* encephalopathy reported in the literature and in the present study.

Type	Mutation nomenclature (GRCh37)	Exon	Isoform	Domain	Expected effect	Mutation type	Base change (NM_006772.2)	Amino acid change	Inheritance	Patient ID	Reference
Point	Chr6:g.33393668dup	3	1, 2, 4		Hap	frameshift	c.283dup	p.His95Profs*5	Mosaic father	Patient 1	Berryer et al, 2013
Point	Chr6:g.33399963_33399966del	4	1, 2, 3, 4		Hap	frameshift	c.321_324del	p.Lys108Valfs*25	de novo	R0038372	Hamdan et al., 2011
Point	Chr6:g.33399963_33399966del	4	1, 2, 3, 4		Hap	frameshift	c.321_324del	p.Lys108Valfs*25	de novo	T19988	Carvill et al., 2013
Point	Chr6:g.33399975del	4	1, 2, 3, 4		Hap	frameshift	c.333del	p.Lys114Serfs*20	de novo	217-14271-3940	O'Roak et al., 2014
Point	Chr6:g.33399990C>A	4	1, 2, 3, 4		Hap	nonsense	c.348C>A	p.Tyr116*	de novo	Patient #2	von Stüpnagel et al., 2015; this study
Point	Chr6:g.33400460A>T	intron 4	1, 2, 3, 4		Hap	splice	c.388-2A>T	p.?	de novo	T15924	Carvill et al., 2013
Point	Chr6:g.33400477C>T	5	1, 2, 3, 4		Hap	nonsense	c.403C>T	p.Arg135*	de novo	Patient #3	This study
Point	Chr6:g.33400486A>T	5	1, 2, 3, 4		Hap	nonsense	c.412A>T	p.Lys138*	de novo	R0033401	Hamdan et al., 2009
Point	Chr6:g.33400501C>T	5	1, 2, 3, 4		Hap	nonsense	c.427C>T	p.Arg143*	de novo	T22387	Carvill et al., 2013
Point	Chr6:g.33400501C>T	5	1, 2, 3, 4		Hap	nonsense	c.427C>T	p.Arg143*	de novo	Patient #4	This study
Point	Chr6:g.33400505_33400508del	5	1, 2, 3, 4		Hap	frameshift	c.431_434del	p.Thr144Serfs*29	de novo	259214	Parker et al., 2015
Point	Chr6:g.33400529_33400533del	5	1, 2, 3, 4	PH	Hap	frameshift	c.455_459del	p.Arg152Glnfs*14	de novo	Patient #5	This study
Point	Chr6:g.33400564C>T	5	1, 2, 3, 4	PH	Hap	nonsense	c.490C>T	p.Arg164*	de novo	Patient #6	This study
Point	Chr6:g.33400583G>A	5 (last nucleotide)	1, 2, 3, 4	PH	Hap	missense +splice	c.509G>A	p.Arg170Gln	de novo	259840	Parker et al., 2015
Point	Chr6:g.33400584G>T	intron 5	1, 2, 3, 4	PH	Hap	splice	c.509+1 G>T	p.?	de novo	Patient #7	This study
Point	Chr6:g.33402928G>A	intron 5	1, 2, 3, 4	PH	Hap	splice	c.510-1G>A	p.?	de novo	Patient 16	De Ligt et al., 2012
Point	Chr6:g.33403326G>A	7	All	PH	mis	missense	c.698G>A	p.Cys233Tyr	de novo	12804.p1	O'Roak et al., 2014
Point	Chr6:g.33405482G>A	8	All	C2	Hap	nonsense	c.800G>A	p.Trp267*	de novo	T15923	Carvill et al., 2013
Point	Chr6:g.33405510dup	8	All	C2	Hap	frameshift	c.828dup	p.Lys277Glnfs*7	NA	Patient #8	This study
Point	Chr6:g.33405662T>C	8	All	C2	mis	missense	c.980T>C	p.Leu327Pro	de novo	LEM300468 + LEM300469	Parker et al., 2015
Point	Chr6:g.33405725_33405726del	8	All	C2	Hap	frameshift	c.1043_1044del	p.Val348Alafs*70	de novo	Patient 8	Vissers et al., 2010
Point	Chr6:g.33405739del	8	All	C2	Hap	frameshift	c.1057del	p.Leu353Trpfs*13	NA	Patient #9	This study
Point	Chr6:g.33405766T>A	8	All	C2	mis	missense	c.1084T>A	p.Trp362Arg	de novo	Patient 2	Berryer et al, 2013
Point	Chr6:g.33405935_33405936del	8	All	RASGAP	Hap	frameshift	c.1253_1254del	p.Lys418Argfs*54	de novo	ER53899 and Patient #10	Rauch et al., 2012; this study
Point	Chr6:g.33406572_33406575del	10	All	RASGAP	Hap	frameshift	c.1552_1555del	p.Tyr518Asnfs*8	de novo	259041	Parker et al., 2015
Point	Chr6:g.33406650C>T	10	All	RASGAP	Hap	nonsense	c.1630C>T	p.Arg544*	de novo	Patient #11	This study
Point	Chr6:g.33408514C>T	11	All	RASGAP	mis	missense	c.1685C>T	p.Pro562Leu	de novo	Patient 3	Berryer et al, 2013
Point	Chr6:g.33408514C>T	11	All	RASGAP	mis	missense	c.1685C>T	p.Pro562Leu	de novo	Patient #12	This study
Point	Chr6:g.33408564C>T	11	All	RASGAP	Hap	nonsense	c.1735C>T	p.Arg579*	de novo	R0032180	Hamdan et al., 2009



Point	Chr6:g.33408612del	11	All	RASGAP	Hap	frameshift	c.1782del	p.Leu595Cysfs*55	de novo	212-21043-1	O'Roak et al., 2014
Point	Chr6:g.33408652_33408653del	11	All	RASGAP	Hap	frameshift	c.1823_1824del	p.Phe608Trpfs*9	de novo	13073.p1	O'Roak et al., 2014
Point	Chr6:g.33409031T>A	12	All	RASGAP	Hap	nonsense	c.1995T>A	p.Tyr665*	NA	Patient #13	This study
Point	Chr6:g.33409140C>T	12	All	RASGAP	Hap	nonsense	c.2104C>T	p.Gln702*	de novo	T2528	Carvill et al., 2013
Point	Chr6:g.33409426del	13	All	RASGAP	Hap	frameshift	c.2184del	p.Asn729Thrfs*31	de novo	Patient 5	Berryer et al, 2013; Dymment et al, 2015
Point	Chr6:g.33409454_33409455del	13	All		Hap	frameshift	c.2212_2213del	p.Ser738*	de novo	Patient 4	Berryer et al, 2013
Point	Chr6:g.33409456_33409459del	13	All		Hap	frameshift	c.2214_2217del	p.Glu739Glyfs*20	de novo	Patient #14	This study
Point	Chr6:g.33409537G>A	intron 13	All		Hap	splice	c.2294+1G>A	p.?	de novo	R0034526	Hamdan et al., 2011; Xiong et al., 2015
Point	Chr6:g.33410767del	15	All	SH3	Hap	frameshift	c.2438del	p.Leu813Argfs*23	de novo	R0033475	Hamdan et al., 2009
Point	Chr6:g.33410959dup	15	All		Hap	frameshift	c.2630dup	p.Thr878Aspfs*60	de novo	BO14/09	Rauch et al., 2012
Point	Chr6:g.33411006del	15	All		Hap	frameshift	c.2677del	p.Gln893Argfs*184	de novo	R0034759	Hamdan et al., 2011
Point	Chr6:g.33411093C>T	15	All		Hap	nonsense	c.2764C>T	p.Arg922*	de novo	264135	Parker et al., 2015
Point	Chr6:g.33411103del	15	All		Hap	frameshift	c.2774del	p.Leu925PProfs*152	de novo	259606	Parker et al., 2015
Point	Chr6:g.33411111C>T	15	All		Hap	nonsense	c.2782C>T	p.Gln928*	de novo	258913	Parker et al., 2015
Point	Chr6:g.33411262del	15	All		Hap	frameshift	c.2933del	p.Pro978Hisfs*99	de novo	Patient #15	This study
Point	Chr6:g.33411606C>T	15	All		Hap	nonsense	c.3277C>T	p.Gln1093*	de novo	Pat. 8 "258536"	Parker et al., 2015
Point	Chr6:g.33411735dup	15	All		Hap	frameshift	c.3406dup	p.Gln1136Profs*17	NA	Patient #16	This study
Point	Chr6:g.33411738G>A	intron 15	All		Hap	splice	c.3408+1G>A	p.?	de novo	Patient #17	This study
Point	Chr6:g.33414346G>A	intron 16	All	CC	Hap	splice	c.3583-6G>A	p.Val1195Alafs*27	de novo	APN-139	Redin et al., 2014

Type	Mutation nomenclature (GRCh37)	Type				Size	Genes altered	Consequence	Inheritance	Reference	
CNV	Chr6:33389736-33406339	Deletion	All	PH, C2, RASGAP	Hap	16.6 Kb	SYNGAP1 (intron 1 - exon 9)	p.?	de novo	Patient #1	This study
CNV	Chr6:33356364-33406339	Deletion	All	PH, C2, RASGAP	Hap	50 Kb	SYNGAP1 (5'UTR-exon 9) + 3 others	p.?	de novo	Case report	Writzl et al., 2013
CNV	Chr6:33291871-33404064	Deletion	All	PH, C2, RASGAP	Hap	112 Kb	SYNGAP1 ('UTR-intron8) + 5 others	absence of protein synthesis	de novo	Case report	Pinto et al., 2010
CNV	NA	Deletion	All	All	Hap	300 Kb	Entire gene + 6 others	absence of protein synthesis	de novo	Case report	Zollino et al., 2011
CNV	Chr6:33201710-33595089	Deletion	All	All	Hap	393 Kb	Entire gene + 18 others	absence of protein synthesis	de novo	Case report	Parker et al., 2015

CNV	Chr6:33273955–34086729	Deletion	All	All	Hap	813 Kb	Entire gene + 18 others	absence of protein synthesis	de novo	Case report	Krepischi et al., 2010
CNV	t(6;22)(p21.32;q11.21)	Balanced translocation					Interrupts SYNGAP1	p.?	de novo	Case report	Klitten et al., 2013

Hap: haploinsufficiency.

Supplementary Table 3

Reference				
<b>Patient</b>	259041	259840	258913	264135
<b>Sex</b>	F	F	F	F
<b>Age (in years)</b>	7	8	7	3
<b>Developmental/neurological evaluation</b>				
<b>Evaluation of developmental delay/intellectual disability</b>	moderate	moderate	moderate	moderate
<b>Age of sitting</b>	20 m	7 m	12 m	24 m
<b>Age of walking</b>	24 m	36 m	60 m	does not walk
<b>Evaluation of speech</b>	50 words, two-word sentences	single words	single words	no speech
<b>Neurological signs</b>	unsteady gait	wide-based gait	wide-based gait	NA
<b>OFC</b>	-1.6 SD	+ 0.8 SD	- 2.6 SD	- 2.5 SD
<b>Behavior</b>	aggressiveness, routine-orientated	autism, aggressiveness, routine-orientated, obsessions	aggressiveness, routine-orientated, stereotypies	autism, aggressiveness, obsessions
<b>Brain MRI</b>	NA	normal	normal	normal
<b>Epilepsy</b>	no epilepsy	yes	yes	yes
<b>Epilepsy onset</b>	NA	6 y	2 y	2 y
<b>Seizure types</b>	NA	mj, abs	mj, abs, drop at	abs, drop at
<b>Epilepsy outcome</b>	NA	NA	NA	NA
<b>EEG</b>	NA	NA	NA	NA

NA=not available or not applicable; F= female; M=male; m= months; y=years; ADHD= ;  
**Seizures:** a = aura; abs = absences; atyp abs = atypical absences; drop at = drop attack  
**EEG:** DS= diffuse slowing; ETPs= epileptic potentials; GPSW= generalized poly spike

Parker et al. Am J Med Genet 2015				
259214	259606	258536	Pat. 8 "258536"	LEM300469
M	F	F	F	M
8	12	5	8	14
moderate	moderate	moderate	moderate	severe
15 m	NA	7 m	12 m	24-36 m
17 m	24 m	19 m	30 m	> 60 m
200 single words	20 single words	2-word sentences	4-word sentences	absent
NA	wide-based gait	unsteady gait	NA	ataxic gait
- 1.1 SD	- 1.83 SD	- 2.9 SD	0 SD	- 0.98 SD
autism, aggressiveness, obsessions	aggressiveness, stereotypies	autism, aggressiveness, obsessions	ASD	autism, laughter outbursts, routine-orientated, obsessions
normal	normal	NA	NA	normal
no epilepsy	yes	no epilepsy	yes	yes
NA	3 y	NA	5 y	13 m
NA	head drops & blinking, PS	NA	abs, drop at	FS, abs, drop at, occasional tc, mj
NA	NA	NA	NA	NA
NA	NA	NA	NA	NA

attention deficit hyperactivity disorder; ASD = autistic spectrum disorder; EE= epileptic ks; FDS = focal discognitive seizures; FS = febrile seizures; GTCS = generalized tonic waves; GSW= generalized spike waves; MFD= multi focal discharges; GS=generalize

	Redin et al. J Med Genet 2014	Carvill et al. Nat Genet 2013				
LEM300468	APN-139	T15923	T22387	T19988	T15924	T2528
M 14	M 6	F 26	F 7	M 18	M 11	M 26
severe	moderate	severe	severe	moderate	severe	moderate
24-36 m	delayed	NA	NA	NA	NA	NA
> 60 m	delayed	NA	NA	NA	NA	NA
absent	absent	NA	NA	NA	NA	NA
ataxic gait	hypotonia, cerebellar syndrome	NA	NA	NA	NA	NA
- 1.2 SD	NA	NA	NA	NA	NA	NA
autism, laughter outbursts, routine- orientated, obsessions	stereotypic movements, hetero and auto- aggressiveness	ASD	ASD	NA	ASD	NA
normal	NA	NA	NA	NA	NA	NA
yes	NA	yes	yes	yes	yes	yes
13 m	NA	36	10	NA	6	18
FS, abs, drop at, occasional tc, mj	NA	atyp abs, a, FDS, mj	abs, mj	FDS	abs, tc	FS; abs, a, FDS, mj, tc, NCS
NA	NA	EE	EE	EE	EE	EE
NA	NA	SSW, MFD	GSW	MFD, DS	GSW, MFD, GPSW	SSW, bioccipital ETPs, DS

; encephalopathy; EEG= electro encephalogram; mod.= moderate; MRI= magnetic resonance imaging; clonic seizures; myo abs = myoclonic absence; myo at = myoclonic atonic; mj = myoclonic jerks; PC and spikes; GSW=slow waves; poly-SW= poly spike waves; SSW= slow spike waves

Berryer et al. Hum Mutat 2013					de Light et al. New Engl J Med 2012	Hamdan et al. J Child Neurol 2012
Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 16	Patient 1 R0034759
F	M	F	M	F	M	F
16	3.5	4.3	2.5	9.3	7.7	3.7
moderate	moderate	mild	moderate	moderate/severe	moderate	moderate/severe
6 m	NA	NA	9 m	NA	12 m	NA
10.5 m	30 m	15 m	21 m	26 m	22 m	22 m
impaired	absent	delayed	absent	impaired	absent	absent
none	hypotonia	none	hypotonia	ataxic gait	NA	hypotonia
normal	normal	normal	"microcephaly"	normal	normal	normal
recurrent seasonal depression	ASD	autism, irritability, automutilations, sleeping difficulties	sleeping difficulties and aggressiveness	ASD	self-mutilation, inappropriate laughters	attention deficit; aggressive adverse behavior
normal	normal	NA	normal	normal	normal	normal
yes	yes	no epilepsy	yes	yes	yes	yes
18	30	NA	36	38	60	29
myo abs, abs, mj	drop at, abs	NA	NA	drop at, abs	NA	head drop, abs
poor control	poor control	NA	NA	good control	NA	good control
GSW posterior predominance	GSW posterior predominance	"intermittent and slow dysfunction in the occipital regions"	bursts of GS + GSW bioccipital predominance	"abnormal bursts, in the right vertex region, of poorly formed waves during sleep"	NA	GSW posterior predominance

mg; TPM = topiramate; VPA = valproic acid; OFC=occipitofrontal  
 S = partial complex seizures; tc = tonic-clonic; PS: photosensitivity

et al. Biol Psychiatr 2011		Hamdan et al. Am J Hum Genet 2011, New Engl J Med 2009 and Biol Psychiatr 2011			Vissers et al. Nat Genet 2010	Rauch et al. La
Patient 2 R0038372	Patient 3 R0034526	Patient 1 R0033401	Patient 2 R0032180	Patient 3 R0033475	Patient 8	BO14/09
M 4	M 13	F 4.4	F 5.8	F 12	F NA	F 11
moderate/severe	moderate/severe	moderate	moderate	moderate	mild/moderate	moderate/severe
NA	NA	NA	NA	NA	19 m	15 m
17 m	24 M	24 M	21 m	24 m	NA	24 m
impaired	impaired	impaired	impaired	impaired	absent	absent
normal	normal	hypotonia	hypotonia	normal	hypotonia	NA
normal	normal	normal	NA	NA	normal	normal
ADHD; aggressiveness; temper tantrums	autism, mood instability, temper tantrums	no ASD	no ASD	no ASD	NA	auto-aggressive behaviour
mildly enlarged ventricles	NA	normal	normal	normal (TDM)	mild myelination delay (10 m)	normal
yes	no epilepsy	yes	yes	no epilepsy	yes	yes
24	NA	15	28	NA	48	26
FS, mj,abs	NA	febrile and afebrile GTCS; PCS	myo at	NA	NA	abs, atonic seizures
good control	NA	good control by TPM	good control by VPA	NA	NA	NA
bioccipital SSW	NA	bioccipital spikes during light stimulation	bioccipital spikes during light stimulation	NA	NA	NA

Incet 2012	Klitten et al. Epilepsia 2011	Writzl et al. Am J Med Genet 2013	Zollino et al. Eur J Hum Genet 2011	Krepischi et al. Am J Med Genet 2010
ER53899				
M 5	M 25	M 9	F 5	M 6.8
severe	severe	moderate	severe	moderate
17 m	NA	10 m	NA	6.5 m
36 m	36 m	29 m	NA	16 m
absent	impaired	impaired	impaired	impaired
hypotonia	NA	NA	normal	NA
normal	NA	normal	normal	normal
ADS, stereotypies	ASD	none	ASD, stereotypies	NA
arachnoid cyst	NA	normal	normal	NA
NA	yes	yes	yes	NA
NA	13	48	3	NA
NA	abs, myo abs, atyp abs, drop at with mj	abs, head nodding	myo at,	NA
NA	poor control	good control VPA	positive effect by VPA and TPM	NA
NA	GSW, GPSW	MFD	MFD;SSW; Sp and Poly-SPW; subcontinuous during sleep and were reminiscent of the EEG features of the Lennox-Gastaut syndrome	NA



**summary**

n=35

sex ratio M/F : 0.84  
mean 10 y

mild 1; mild/moderate 1;  
moderate 20;  
moderate/severe 5, severe 8

mean 14.8 m, median 12 m

mean 25.3 m; median 24 m

absent speech 11; single  
words 4; 2-4-word sentences  
3; "impaired" 12

none 6; unsteady gait/ataxia  
8; hypotonia 8

normal 22; microcephaly ( $\leq$  -  
2.5 SD) 6

ASD 20; aggressiveness 11;  
stereotypies 5

normal 19; minor nonspecific  
findings 3

yes 26; no 6

mean 30 m; median 24 m;  
75th centile 3.5 years

abs 19; mj 10; drop at 8; tc 4;  
FDS/PCS 4; myo abs 2;  
GTCS 1; PS 1

NA 20; EE 5; poor control 3;  
good control 7

occipital predominance of  
anomalies 8; PS 2