

ORIGINAL ARTICLE

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

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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 centres were also collected, focusing on developmental aspects and the associated epilepsy 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 behaviours in eight 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, characterised by falls or head drops due to atonic or myoclonic seizures, (myoclonic) absences and/or eyelid myoclonia. Triggers of seizures were frequent (n=7). 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.

Conclusions *SYNGAP1* encephalopathy is characterised by early neurodevelopmental delay typically preceding the onset of a relatively recognisable epilepsy comprising generalised seizures (absences, myoclonic jerks) and frequent triggers.

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 N-Methyl-D-aspartic acid (NMDA) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors at the glutamatergic synapses and mediate signalling downstream of glutamate receptor activation.^{3–4} While complete *Syngap1* deficiency in mice is lethal at early postnatal stages, heterozygous *syngap1* +/- mice are viable but show behavioural and cognitive disturbances.^{5–8} *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 behavioural 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^{11–15} or direct

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sequencing of the *SYNGAP1* gene through a candidate gene approach.^{16–24} Recently, seven *SYNGAP1* mutations were identified by exome sequencing in a series of 1133 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 27–30} 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 analysed 251 patients with variable neurodevelopmental phenotypes including ID, EE and ASD (see online 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 hybridisation (array-CGH). Clinical and molecular data of 13 additional patients with *SYNGAP1* mutation, identified in 12 other centres, were collected: all patients with a mutation introducing a premature termination codon or occurring de novo (ie, 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, behavioural and epilepsy history, including EEG and imaging data if available. Most patients were evaluated according to developmental scales routinely used in enrolled centres by clinicians trained in neurodevelopment or neuropsychologists (eg, Brunet-Lezine, HAWIK-IV or SON-R2 scales). The sex ratio was eight males/nine females. Mean age at the time of the study was 10.3 years (range 3–29 years).

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 50 Mb 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 Integrigen 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 GATK³³ 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 <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 (Integrigen 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, 48×48 Access Arrays) and sequenced on a MiSeq Illumina sequencer as paired-end 2×250 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 analysing 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 with 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 visualised 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 2 cases published previously^{12 24} and 11 additional patients with *SYNGAP1* mutations identified in other centres (see [table 1](#) and online supplementary table S2).

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 twins²³) carrying 43 different point mutation or indels limited to the *SYNGAP1* gene have been described to date ([figure 1](#) and online supplementary table S3). Three recurrent mutations (c.321_324del, c.427C>T/p.Arg143*, c.1685C>T/p.Pro562Leu)

Table 1 Molecular and clinical data from the 17 patients with *SYNGAP1* mutations*

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	De novo	De novo	De novo	De novo	De novo	De novo	De novo	Parents not tested	Parents not tested
Level of intellectual disability/age at evaluation	Severe/10 years	Mild/12 years	Moderate/5.5 years	Severe/10.8 years	Severe/11 years	Severe/11 years	Moderate/5 years	Moderate/4.5 years	Moderate/5.5 years
Developmental stages									
Age of sitting/walking	7 months/24 months	10 months/<18 months	10 months/20 months	10 months/24 months	16 months/36 months	8 months/20 months	10 months/22 months	9 months/15 months	NA/24 months
Age of first words/first sentences	4 years/no sentences	14 months/NA	33 months/no sentences	5 years (5 words)/no sentences	4 years transient 'mama' 'papa'/no sentences	NA/no sentences	3 years/5 years	23 months	36 months/no sentences
Current language ability	Single words	NA	~50 words	10 words	Absence of speech	Few words at 11 years	5-word sentences	Short sentences	15 words
Regressive episode during the development/age	Slowing of development with untreated epilepsy/2 years	No	No	No	Possible (loss of few acquired words)	No	Loss of few dissyllable words after 20 months	No	NA
Autism spectrum disorder	No	Yes	No	Yes	Yes	Yes	No	No	No
Clinical examination									
Age at examination	14 years	12 years	5.5 years	10.8 years	11 years	10 years	5 years	6 years	5.5 years
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)
Neurological 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
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	

Continued

Table 1 Continued

Patient ID	10	11	12	13	14	15	16	17	Summary
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	De novo	De novo	De novo	Parents not tested	De novo	De novo	Parents not tested	De novo	
Level of intellectual disability/age at evaluation	Severe/4 years	Severe/3 years	Severe/22 years	Severe/12 years	Mild/8 years	Moderate/5 years	Severe/8.5 years	Severe/10 years	Mild n=2; moderate n=5; severe n=10/mean age at evaluation 8.7 years
Developmental stages									
Age of sitting/walking	15–18 months/36 months	12 months/walks only with aid	12 months/38 months	NA/36 months	8 months/18 months	10 months/18months	16 months/30 months	25 months/4.5 years	Mean 12 months/27.7 months
Age of first words/first sentences	~29 months transient ‘mama’, ‘papa’/no sentences	3 years ‘papa’ only/no sentences	No words/no sentences	No words/no sentences	12 months/6 years	3 years/no sentences	17 months/no sentences	No words/no sentences	Mean age first words 2.6 years
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	12 months—with febrile seizures	No	14 months	No	Loss of words at age 18–30 months	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 years	3 years	22 years	12 years	8 years	7 years	8.5 years	6.6 years	Mean 8.9 years
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
Neurological 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; OFC, occipitofrontal circumference.

were found in two patients each. Pathogenic mutations in *SYNGAP1* are distributed throughout the gene, especially in exons 5, 8 and 15, which are among 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

All patients with *SYNGAP1* anomalies of our series had ID, which was evaluated as severe in 10 patients, moderate in 5 and mild in 2 (see table 1 and online supplementary table S1). 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). Also, 10/17 patients could walk by age 2 years and 14/17 by age 3 years. All patients had speech delay: 12 of them spoke first words at a mean age of 2.5 years and 5 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 behavioural anomalies were the most prominent features.

In total, 8 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 (see online supplementary table S1). 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 stereotypes (n=10), temper tantrums, aggressiveness, self-injurious behaviour 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 4. Some patients had orthopaedic 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 myoclonic astatic epilepsy (MAE, ie, Doose syndrome) and epilepsy with myoclonic absences (EMAs) was made in three and one patients, respectively. The others were diagnosed with unclassified genetic generalised epilepsy (GGE). None had a diagnosis of Lennox–Gastaut syndrome (LGS).

The epilepsy responded to a single antiepileptic drug (AED), mostly sodium valproate, in seven patients and was pharmacoresistant in nine (list of AEDs is reported in table 2). 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 patient 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 pharmacoresistant (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 generalised (n=13), generalised with a posterior predominance or posterior only (n=5). Paroxysmal anomalies were localised 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 epilepsy 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, generalised epilepsy in most and autistic behaviour in a half of them (see online supplementary table S3). 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 orthopaedic issues.

Occipitofrontal circumference 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

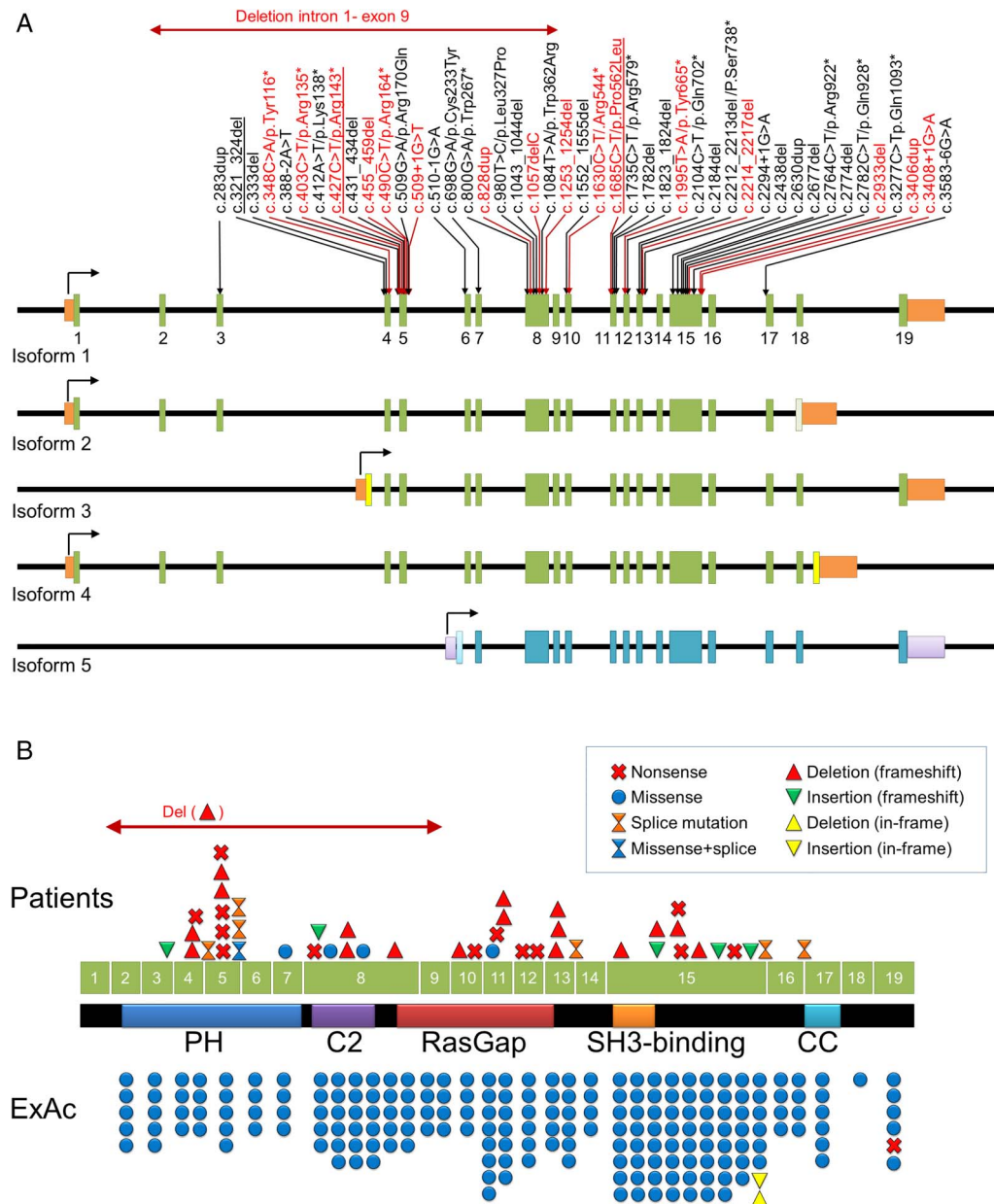


Figure 1 Summary of *SYNGAP1* mutations identified in this study and the literature. (A) Location of mutations on the different *SYNGAP1* isoforms. Mutations in red correspond to the patients identified in this study. Mutations in black correspond to previously published patients. Recurrent mutations are underlined. Isoform 1 corresponds to the longest isoform (NM_006772.2, N-terminus: SYNGAP A, C-terminus: SYNGAP α 2); isoform 2 is obtained through alternative splicing of exons 18 and 19 and differs in its C-terminus (SYNGAP β : 1265–1343: RLMLVEEELR...NGEFRNTADH \rightarrow SPSLQADAGGGAAPGPPRHG); 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 MGLRPPTSP...RRCSSCCFP); isoform 4 is obtained through alternative splicing of exon 19 and differs in its C-terminus (SYNGAP γ : 1296–1343: ERQLPLPGTNPVR...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 α 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.

acquired a few words between 1 and 4 years old but only three patients were able to speak simple sentences, which is likely related to ID. 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 epilepsy 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

Table 2 Epilepsy features in SYNGAP1-related encephalopathy

Patient ID	1	2	3	4	5	6	7	8	9
Age at seizure onset	24 months	24 months	22 months	4 years	3 years	30 months	5 years	33 months	30 months
Seizure type at onset	Myoclonic jerks (falls)	Drop attacks	Febrile seizure	GTCS, abs.	Tonic febrile and afebrile, myoclonic jerks	Not defined	Abs.	Abs.	Head nodding, abs.
Seizure types during disease course	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)
Epilepsy syndrome	EMA	MAE 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
Febrile seizures	No	Yes	Yes	No	Rare	No	No	No	No
Status epilepticus	No	No	No	No	No	No	No	No	No
Frequency of seizures	>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
Lifetime/current antiepileptic treatment	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
Pharmacoresistant EEG	No	No	No	No	Partial	No	No	Yes	Yes
Age at examination	9 years	2-15 years	4.5 years	9 years	1-5 years	3-8 years	5 years	8.5 years	5 years
Main abnormalities	Generalised bursts of S	Generalised PsW and photoconvulsions	Frontal and generalised SpW and PSW	Irregular spike-slow-wave complexes: generalised, maximum frontal; β -waves	1 year: normal; 3.5 years: generalised bursts of S, S+SW in posterior areas; 5 years: slow background activity, frontotemporal 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
Patient ID	10	11	12	13	14	15	16	17	Summary
Age at seizure onset	One seizure at 3.5 years	24 months	12 months	<2 years	5 years	22 months	27 months	8 years	Mean 35.4 m, median age 28.5 months, 75th centile 39 months
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	MAE	Unclassified GGE	MAE	Unclassified GGE	Unclassified	Unclassified 12, MAE 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

Continued

Table 2 Continued

Patient ID	10	11	12	13	14	15	16	17	Summary
Frequency of seizures	Only one until now	Several/day	Several/month	10/day	100/day	Several/day	Several/day	4–8/month	
Lifetime/current antiepileptic treatment	No	VPA	VPA, CBZ, CZP, ZNM/LEV, TPM	VPA, ZNM, LTG	VPA/LEV+ETH	VPA, LTG+VPA, LTG, LEV, CLN, ACTH	VPA, CBZ, TPM/ketogenic diet	VPA	
Pharmacoresistant EEG	Not applicable	No	Yes	Yes	Yes	Yes	Yes	Partial	Yes 9, no 7
Age at examination	1.8 and 2.5 years	3 years	3–8 years	2–10 years	2–5 years	7	8.5 years	2.3 years	
Main abnormalities	1st: SW; 2nd: no abnormalities	Abnormal background, generalised slowing, recorded seizures with eyelid myoclonia and generalised seizure patterns	Bursts of S and SW in the occipital region after eye closure	Generalised SpW	2 years: normal; 5 years: ictal bursts of diffuse PSW with posterior predominance after eyes closer and photic stimulation	Focal SpW in central-parietal areas, generalised S and PSW	Generalised PSW and frontal SW	Multifocal SW	
Triggers of seizures	None	PS	FOS	PS	PS, FOS	None	None	PS	PS 4, FOS 1, PS+FOS 1, other 1

*Epilepsy aggravated.

Abs., absences; ACTH, adrenocorticotrophic hormone; CBZ, clobazam; CLN, clonazepam; CZP, carbamazepine; EMA, epilepsy with myoclonic absences; ETH, ethosuximide; FOS, fixation-off sensitivity; GGE, genetic generalised epilepsy; GTCS, generalised tonic-clonic seizures; LEV, levetiracetam; LTG, lamotrigine; MAE, myoclonic astatic epilepsy; NA, not available; OXC, oxcarbazepine; PS, photosensitivity; PSp, polyspikes; PSW, polyspike waves; S, spikes; SpW, spike waves; SW, slow waves; TPM, topiramate; VPA, valproic acid; ZNM, zonisamide.

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 behavioural 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 pharmacoresistant 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 ID²⁵ 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 generalised seizures, like those reported in a previous study,²⁰ only a few of them also experienced focal clonic or tonic clonic seizures. Generalised 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 generalised and/or occipital anomalies on EEG, may guide towards 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 MAE, whose diagnosis has been suggested in three and one 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 mutation²⁰ 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 MAE 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 MAE and 2 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 emphasised. The fixation-off phenomenon has been described once.²⁴ In our series, PS 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

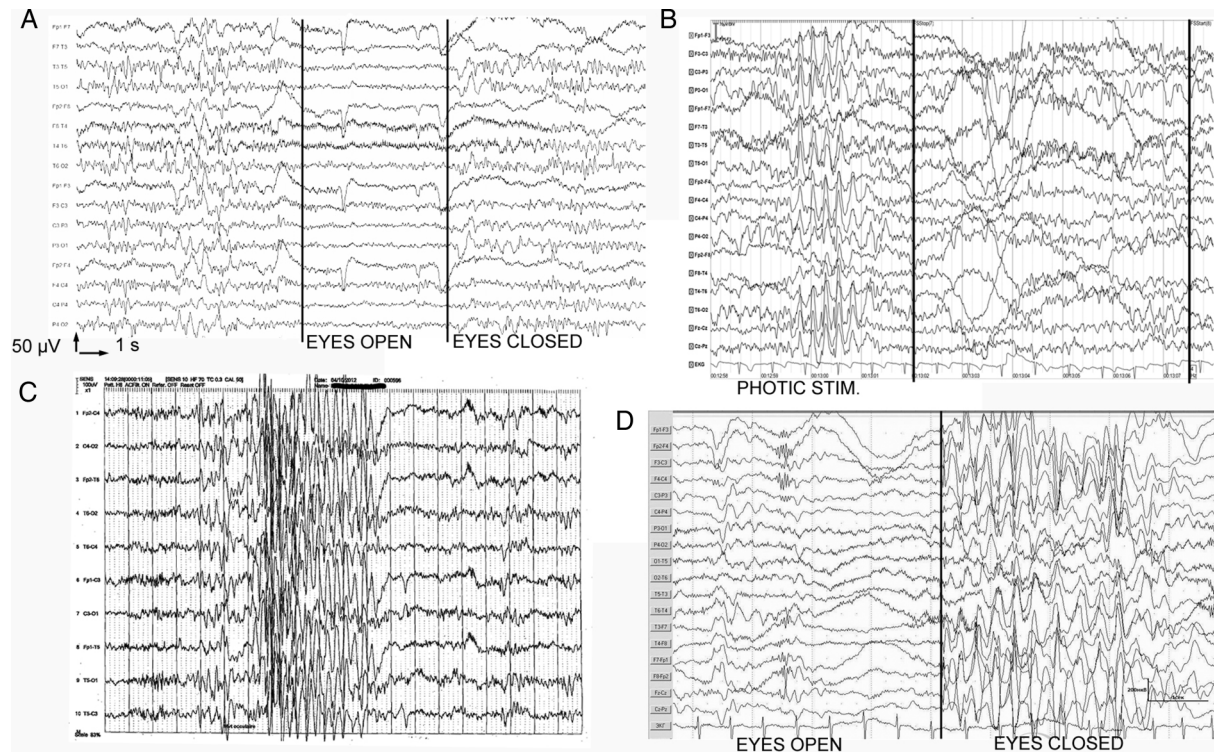


Figure 2 EEG samples from patients exemplifying electroencephalographic findings in *SYNGAP1*-related encephalopathy. (A) Sample demonstrating normalisation of paroxysmal activity by eye opening, that is, fixation-off sensitivity, in patient #2. (B) Sample showing paroxysmal activity under photic stimulation, that is, photosensitivity, in patient #2. (C) Sample from patient #1: burst of generalised spikes concomitant of a rapid eye deviation (fast rhythms are due to benzodiazepine therapy). (D) Sample from patient #12 showing the appearance of generalised spike wave complexes with a low degree of bilateral synchronisation after eye closure (fixation-off phenomenon).

of PS is significant since clinical PS is found in only 10% of patients with epilepsy in the 7–19-year-old group.³⁵ We assume that PS 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 PS, 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, whose 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 (see online supplementary tables S2 and S3), 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 pharmacoresistant 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 PSD.³⁶ The fact that a subset of patients with *SYNGAP1* mutations exhibit autistic behaviours 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

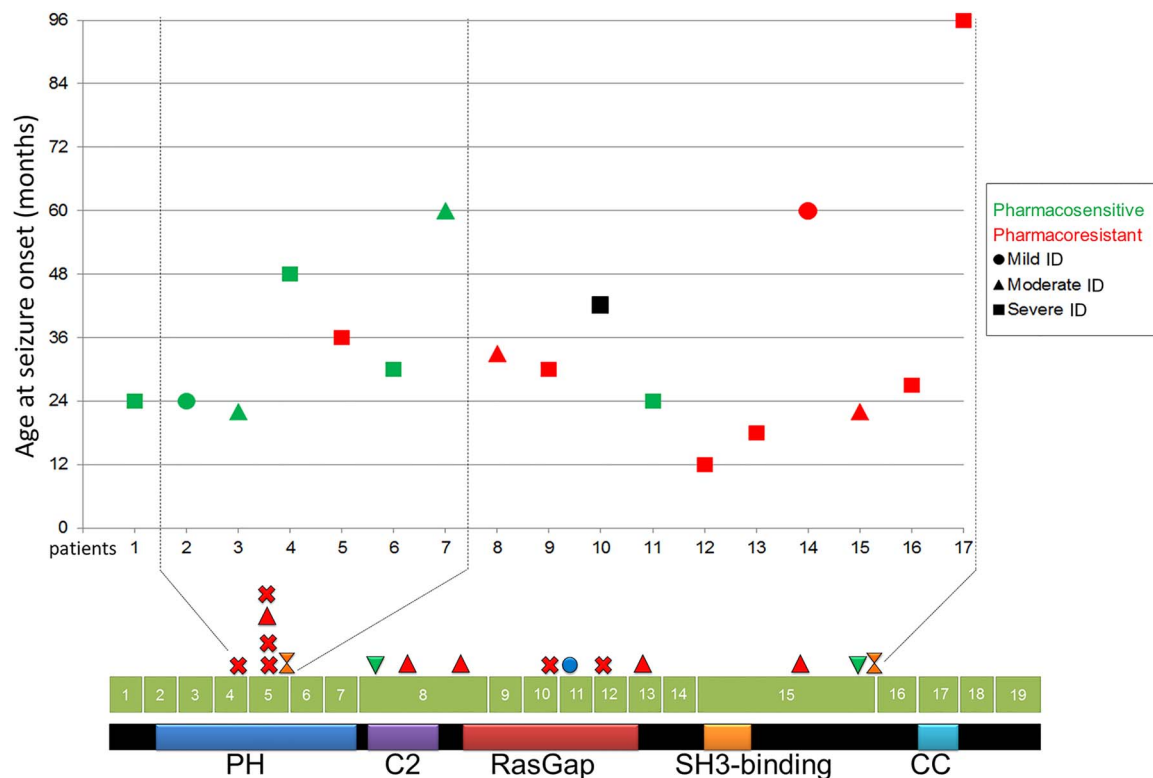


Figure 3 Graphical representation of clinical data (age at epilepsy onset, level of intellectual disability (ID) and pharmacoresistance or pharmacosensitivity) in our patients series. X-axis indicates the number of the patient, ordered by the position of the mutation on the gene, except patient 1, who 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 having epilepsy, was not considered for this analysis. The age at the first seizure is neither related to the resistance or sensitivity of the epilepsy to antiepileptic drug 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 ($p=0.001$).

development impairment. Many genes mutated in patients with ASD and ID are linked with neuronal signalling pathways and may alter the synaptic plasticity underlying the building, refinement and consolidation of neuronal networks associated with learning and adaptive behaviours, 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* haploinsufficiency in mice, that is, behavioural 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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Genetic and neurodevelopmental spectrum of *SYNGAP1*-associated intellectual disability and epilepsy

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