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***IQSEC2 disorder: a new disease entity or a Rett spectrum continuum?***
***Running title: Novel insight into IQSEC2 disorder***

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**Abstract**

***Introduction***

*IQSEC2* mutations are associated with *IQSEC2*-related intellectual disability (ID). Phenotypic spectrum has been better defined in the last few years by the increasing number of reported cases although the genotype–phenotype relationship for *IQSEC2* remains overall complex. As for *IQSEC2*-related ID a wide phenotypic diversity has been described in Rett syndrome (RTT). Several patients harbouring *IQSEC2* mutations present with clinical symptoms similar to RTT and some cases meet most of the criteria for classic RTT.

***Materials and Methods***

With the aim of establishing a genotype-phenotype correlation, we collected data of 16 patients harbouring previously unreported *IQSEC2* mutations and of 5 novel patients carrying CNVs encompassing *IQSEC2*.

***Results and Discussion***

Most of our patients surprisingly shared a moderate-to-mild phenotype. The similarities in the clinical course between our mild cases and patients with milder forms of atypical RTT reinforce the hypothesis that also *IQSEC2* mutated patients may lay under the wide clinical spectrum of RTT and thus *IQSEC2* should be considered in the differential diagnosis. Our data confirm that position, type of variant and gender are crucial for *IQSEC2*-associated phenotype delineation.

**Keywords:** *IQSEC2*, intellectual disability, phenotype-genotype, Rett syndrome

## Introduction

The *IQSEC2* gene (OMIM #300522), mapping on chromosome X, encodes a guanine nucleotide exchange factor for the ADP-ribosylation factor family of GTP-binding proteins. *IQSEC2* is most highly expressed in brain tissues and is implicated in the morphology of developing neurons and dendritic spines[1]. *IQSEC2* was first considered a candidate for X-linked neurologic disorders due to the identification of a *de novo* chromosomal translocation disrupting the gene in a female patient with epileptic encephalopathy[2]. Shortly after, *IQSEC2* mutations were reported in patients with X-linked intellectual disability (ID), seizures, and behavioral and psychiatric abnormalities[3-4].

The increasing number of recently reported cases has helped in defining the wide phenotypic spectrum for this X-linked disorder known as *IQSEC2*-related ID[5,6] and characterized by hypotonia, moderate to severe delayed psychomotor development, ID, poor speech, seizures, autistic features, and stereotypic movements. However, the genotype–phenotype relationship remains to date complex and the simple Mendelian transmission model is not sufficient to explain the observed wide phenotypic variability.

Several female *IQSEC2* mutated patients have been described with clinical symptoms similar to Rett syndrome (RTT), such as language regression, repetitive hand movements, microcephaly, and seizures[7] with some cases meeting most of the criteria, although not all, for classic RTT[8]. Just like in *IQSEC2*-related ID, even in RTT, a wide phenotypic diversity is known. RTT is a clinically defined syndrome characterized by developmental regression followed by stabilization, partial or complete loss of purposeful hand skills and spoken language, gait abnormalities, and stereotypic hand movements[9]. Mutations in *MECP2* (OMIM#312750) have been reported in classic RTT patients but also in a great number of patients with milder forms of atypical RTT, such as the Zappella variant, characterized by a later recovery of speech development[9–11]. A wide phenotypic variability has also recently been described in *FOXG1* mutated patients (# 613454)[12,13].

Here we report the clinical and molecular features of a series of patients harbouring pathogenic *IQSEC2* variants and discuss genotype-phenotype correlations. Most of them surprisingly share a moderate-to-mild phenotype. The similarities in the clinical course between our mild cases and the atypical milder forms of RTT supports the idea that also

*IQSEC2* mutated patients may lay under the wide clinical spectrum of RTT and therefore *IQSEC2* should be considered in the differential diagnosis.

## Materials and methods

### Human subjects

Patients were recruited in five different centers (Italy, Spain, Belgium, Switzerland and Nicaragua) thanks to a collaborative research call of the European Reference Network Ithaca (Intellectual disability, TeleHealth, And Congenital Anomalies). Informed written consent was locally obtained for all families.

### Molecular analysis

Whole exome sequencing (WES) or next generation targeted panels were performed. Genome-wide array-comparative genomic hybridization was used to identify CNVs involving *IQSEC2* gene (see Supporting Information).

## Results

### Clinical description

We collected clinical and molecular data of 21 patients (20 unreported and 1 previously reported[14]) harbouring pathogenic *IQSEC2* variants. Epilepsy is a common characteristic in our cohort, being evident in 70% of patients (14/20). ID is almost always present, with patients having usually a severe or moderate disability. Language is absent in 48% of patients (10/21) and, when present, it is typically limited to a few words or simple sentences; exceptionally we report cases with language articulated in sentences (patients 2, patient 6, patient 7 and his family, patients 9, 13, 14, 15, 20 and 21). Hands stereotypes are described in 43% (9/21) of our patients. A regression in neurodevelopmental milestones, particularly of manual skills, was reported in two unrelated patients (Patient 11 and 18) starting at 5 years and 2 years, respectively. In addition, in two patients (Patients 1 and 7) we could recognize some evidence of regression that led the clinician to consider RTT spectrum diagnosis, either at the clinical evaluation or while collecting the clinical history. More than half of the patients (57%, 12/21)

have an absent or very limited use of hands. Interestingly, microcephaly is not a constant feature and when present OFC is around the 3rd percentile. Overall clinical data are presented in Table 1, Table 2 and Table 3.

Five unrelated patients and three familial cases show a surprisingly mild phenotype (Patients 1, 2, 3, 5, 6, Patient 7 of family 1, Patients 13, 14 and 15 of family 2 and Patients 20 and 21 of family 3); clinical details are provided in Supporting Information. These patients aged between 2 years and 46 years, all presented normal growth parameters at birth. A sort of regression in neurodevelopmental milestones was considered by the clinician in Patient 1 because she showed an initial progression of the language skills that were then lost and in Patient 7 who showed a temporary regression of hand use with loss of eating skills. Apart from two patients (Patients 1 and 5), all the other patients showed some extent of verbal ability from single words to normal verbal IQ. At the last follow-up all patients showed autonomous walking. Epilepsy was reported in Patients 1, 2, 5, 6 and in all members of family 2. Stereotypes of the hands were described in Patient 1, 2, 3 and 6. Shaking movements involving the whole body were noticed along with hyperactivity in patient 1. Gastroesophageal reflux, constipation, longitudinal scoliosis, bruxism, cold extremities, hyperventilation and bloating were described in Patients 1, 2, 3, 5 and 7. Autism spectrum disorder diagnosis was reported in the majority of patients (Patient 1, 2, 3, 5, 7, 20 and 21).

**Molecular characterization**

All 21 patients, except one[14], harboured novel *IQSEC2* variants among which 5 *de novo* frameshift variants; 4 *de novo* stop variants; 1 inherited splicing variant; 3 missense variants, 1 *de novo* and 2 inherited; 3 CNVs, 2 *de novo* and 1 inherited (Family 3) (Tables 1, 2, 3).

Five mutations, all *de novo*, determined the disruption of the C-terminus of the protein: c.4039dup (p.(Ala1347Glyfs*40)) (NM_001111125.3) (Patient 1), c.4110_4111del (p.(Tyr1371Glnfs*15)) (Patient 2), c.3613_3613delC (p.(Leu1205Trpfs*192)) (Patient 3), c.4419_4431del (p.(Ser1474Argfs*)) (Patient 5) and the mutation c.3859C>T (p.(Gln1287*) (Patient 6).

The two inherited missense mutations are the c.4204G>A (p.(Ala1402Thr)) in Patient 7 and the c.1076G>A (p.(Arg359His)) in Family 2. The first causes a substitution occurring at a conserved position with a CADDphred of 21.7 and has a likely damaging effect on protein structure (Patient 7). The variant was inherited by the mildly symptomatic mother and maternal

grandmother. The second determines the substitution of a highly conserved amino acid, located within the IQ domains, EF-hand binding site and P-loop containing nucleoside triphosphate hydrolases. A different amino acid substitution in the same position (c.1075C>T, p.Arg359Cys) has been previously described as pathogenic[4].

The maternally inherited CNV in Patient 19 (male) is a partial intragenic duplication of 0.41 Mb (ChX(GRCh37):g53300438-53341868), likely determining a disruption of the protein. The mildly symptomatic sister and mother shared the same variant. The boy had an additional maternally inherited duplication of 0.55 Mb (ChX(GRCh37):g.139888816-140443674) of uncertain significance, absent in the sister.

**Discussion**

To date, the genotype–phenotype correlation for *IQSEC2* remains not fully understood: females have been described with *de novo* variants and ID, epilepsy, speech deficits and autism spectrum disorder; however, some studies have also previously reported asymptomatic or mildly affected females[16]. According to previous studies, *IQSEC2* turned out not to be subjected to X-inactivation in all tested human tissues[17,18]. The analysis of expression data in patients unexpectedly shows similar *IQSEC2* gene dosage between males and females[19,20] although to date, it is not fully understood what mechanism can account for these similar gene dosages between genders.

Several female patients harbouring *IQSEC2* mutations were described with clinical symptoms overlapping RTT[7] and some cases also meet most of the criteria for classic RTT[8]. Like in *MECP2*-mutated neurons[21], also in *IQSEC2*-mutated neurons a GABAergic circuit up-regulation has recently been reported[22]. In *IQSEC2* deficient mice, Sah et al showed an age-dependent increase in expression of parvalbumin whose expression in cells is regulated by synaptic calcium influx. This data suggests that *IQSEC2* loss might upset excitatory synaptic activity-induced calcium entry. Interestingly, increased parvalbumin cell number and parvalbumin expression has also been reported in mouse models of RTT caused by *CDKL5* and *MECP2* genetic ablation[23]. Moreover, previous data suggests that the *IQSEC2* expression pattern in the brain mirrors the expression profile of *CDKL5*[2], a gene capable of mediating *MECP*2 phosphorylation[24]. Based on these evidences, as previously speculated[8], we can hypothesize that *IQSEC2* could belong to a molecular pathway shared with other RTT-related genes.

Similar to *IQSEC2*-related ID, a wide phenotypic diversity has been described in RTT. A significant prevalence of late-truncating mutations followed by missense mutations has been

previously noted in the Zappella variant of RTT in comparison with classic RTT, while the analysis of the X-inactivation status in blood questioned the role of the X-inactivation in modulating the phenotype[10]. Accordingly, also in *FOXG1* mutated patients, the most severe phenotype is associated with frameshift or nonsense variants in the N-terminal domain and the forkhead domain except conserved site 1; truncating variants affecting the C-terminal domain or missense variants in the forkhead domain, leading to a protein with residual function, have been found in children with milder phenotypes[13]. Thus, since the phenotypic variability observed in *MECP2* or *FOXG1* mutated patients is mostly linked to site and type of mutation, it is reasonable to think that such mechanism could be the one mostly accounting for the phenotypic variability also observed in *IQSEC2* mutated patients. Indeed, according to our data, patients harbouring *de novo IQSEC2* mutations determining the disruption of the C-terminus of the IQSEC2 protein (Supporting Information, Fig. 1) often share a mild phenotype. Just like in the Zappella variant of RTT, in some of our *IQSEC2* patients a period of perinatal normality was evident, followed by a regression phase and subsequently by a slow reacquisition of motor abilities. The stereotyped movements frequently observed in the initial period also decrease over time. Most of the described patients have currently autonomous walking skills. Some of them present a language, although not fluent, but articulated in simple sentences. Therefore, the milder phenotype in our patients may be likely due to the variants location near the 3′ end of the transcript. In support of this hypothesis, the female patients already reported in the literature, lying in the milder end of the spectrum, carry a frameshift variant in the C-terminus of the protein[25–27](Table 1). The milder phenotype observed in patient 7 and in his female relatives, who share a missense variant falling in the C-terminus of the protein along with the phenotypic variability observed among family 3 members, confirms that in *IQSEC2* disorder, variant type and position together with gender are crucial to determine the individual phenotype. Indeed, differently from RTT due to *MECP2* mutations, originally considered lethal or devastating in males, both pathogenic truncating and missense variants in *IQSEC2* were reported in male patients. However, the missense variants are most often inherited from asymptomatic or paucisymptomatic mothers, as observed in family of patient 7, and no males with an entire *IQSEC2* deletion have been reported to date, indicating likely a possible lethal effect[25]. These data indicate that females were globally less affected than males thus suggesting that an accurate dosage of *IQSEC2* is crucial[25].

It is noteworthy that our cohort also includes patients with truncating or missense mutations lying in proximal protein domains and associated with a mild phenotype, namely, Patient 9 and Family 2 members. These cases, together with the female Patient 4, who present

with a severe phenotype despite a late truncating mutation, suggest that in *IQSEC2*-related disorder, other yet unknown mechanisms, including epigenetic modifications, modifiers genes and/or possible skewed X inactivation, could contribute to the wide clinical variability. Although previous studies indicated that *IQSEC2* turned out not to be subjected to X-inactivation in different human tissues[17,18], to our knowledge, no clarity about *IQSEC2* inactivation in relevant brain areas has been already provided. Indeed, biallelic expression of many genes including *IQSEC2* is inconstant depending on the tissue and also possibly during development[20]. Moreover, most genes escaping inactivation were not fully expressed from the inactivated X chromosome, indicating that the escape is often partial and incomplete[17].

Mignot et al[25] found no obvious correlation between the XCI status and the severity of the phenotype in six *IQSEC2* affected females; since the expression of *IQSEC2* is similar in males and females[19,20], regulatory mechanisms other than XCI may regulate *IQSEC2* dosage in females[20].

The similarities in the clinical course between our cases and patients with milder forms of RTT are in line with the hypothesis that also *IQSEC2* mutated patients may lay under the wide clinical spectrum of RTT. Indeed, most of our cases meet many criteria, although not all, for RTT diagnosis. Among our *IQSEC2* mutated patients, epilepsy is the most common sign, with 70% of them having seizures usually starting after 12 months of age. Epilepsy is an almost constant sign in the RTT-spectrum: in *MECP2*-mutated patients, onset of seizures may vary in age, but they are a very frequent sign; patients harbouring *CDKL5* and *FOXG1* mutation usually have an early-onset epilepsy (generally within the first two years of life, with *FOXG1* mutated patients having onset of seizures ranging from 2 days to 14 years), which can be often drug-resistant[11]. However, it is noteworthy that some signs carefully annotated can allow a differential clinical diagnosis orienting towards a specific molecular entity. Although postnatal microcephaly is quite constant in the RTT-spectrum, interestingly the head circumference of our *IQSEC2* mutated patients was almost always within the normal range. In addition, stereotypic hand movements observed in *IQSEC2* mutated patients, slightly differ from midline typical RTT hand movements or *FOXG1* related tongue stereotypes. Although the absence of language is very frequent in RTT, we reported cases with some skills, albeit limited, of language. A regression phase, similar to what is observed in RTT, is present only in two patients of our cohort, with some patients showing a sort of regression with characteristics that do not fully match those of the criteria for classic RTT[8]. Although any loss of skills could be the result of seizure activity rather than an underlying regression, in all our *IQSEC2* mutated patients showing both evidence of loss of skills and epilepsy, the time between the onset of

regression to the onset of seizures ranges from 7 months to 7 years, making thus unlikely the hypothesis of a regression as result of seizure activity.

Although to date, the presence of dysmorphisms has never been associated with an *IQSEC2*-mutation, in patient 7 we surprisingly noted some remarkable dysmorphisms (large upper incisors, asymmetric anterior downsweep of hairline and large ears). We cannot exclude that these dysmorphisms can be linked to further possible genetic alterations present in the patient and future studies on larger case series of patients are needed to more clearly elucidate a possible association of dysmorphic traits with *IQSEC2* mutations. The clinical picture of our patients, expanding the complex phenotype associated with *IQSEC2* mutations, provides clinicians useful elements for the differential diagnosis of patients likely laying in the RTT-spectrum. Further delineation of the genotype-phenotype correlation through expansion of cases or biological models of disease are needed to better define the *IQSEC2* associated phenotype and to establish a definitive genotype-phenotype correlation.

**Ethical Statement**

The patients and their families gave informed consent for diagnostics testing and research studies including WES (Ethics committee protocol n. 10519, approved on 06-04-2020). Studies were performed and samples were obtained in accordance with the Helsinki Declaration of 1964, as revised in October 2013 in Fortaleza, Brazil.

**Data Availability Statement**

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study

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Table 1. Clinical features of patient 1-7 compared to the previously reported patients with an *IQSEC2* mutation lying in the C-terminus of the protein (Barrie et al, 2020; Radley et al, 2019; Shoubridge et al., 2019, Mignot et al, 2019; Zou et al, 2019)

| ***Features*** | ***Patient 1*** [14] | ***Patient 2*** | ***Patient 3*** | ***Patient 4*** | ***Patient 5*** | ***Patient 6*** | ***Patient 7 (Family 1** including mother and maternal grandmother with very mild symptoms **)*** | ***Barrie et al, 2020*** | ***Radley et al, 2019*** | | | | ***Shoubridge et al, 2019*** | ***Mignot et al, 2019*** | ***Zou et al, 2019*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | ***patient 6*** | ***patient 7 (sister of patient 6)*** | ***patient 13*** | ***patient 14 ( brother of patient 13)*** | | | |
| ***sex*** | F | F | F | F | F | F | M | F | F | F | M | M | F | F | M |
| ***age (years)*** | 8 | 8 | 8 | 21 | 11 | 2 | 12 years and 10 months | 9 | 10 | 10 | 9 | 14 | n.a. | 11 | 2 |
| ***pregnancy issues*** | | threats of abortion | not | not | normal pregnancy, delayed birth at 40weeks of gestation | breech position | polyhydramnios, born at 41 weeks | n.a | caesarean section at 32 weeks' gestation due to intrauterine growth restriction and suspected twin-to twin transfusion syndrome | caesarean section at 32 weeks' gestation due to intrauterine growth restriction and suspected twin-to twin transfusion syndrome | not | increased nuchal translucency | n.a. | intrauterine growth retardation | born at 40+5 weeks by cesarean with a history of intrauterine hypoxia |
| ***auxological parameters at birth (OFC, weight, length)*** | OFC 34 cm (29th percentile, -0.54 SD), weight 3.3 Kg (41st percentile, -0.24 SD) | OFC 34.5 cm (75-90th percentile), weight 3.14 Kg (25-50 ° percentile), height 49 cm (50-75° centile) | OFC 34.7cm (47th percentile, -0.07 SD), weight 3.45 Kg (52th percentile, +0.05 SD), | OFC 36cm (75th percentile, +0,67SD), weight 4 Kg (90th percentile, +1,25SD), height n.a | OFC 33 cm (11th percentile); weight 3.04 Kg (21st percentile); height 50 cm (58th percentile) | OFC 35 cm (54th percentile); weight 2.65 Kg (7th percentile); height 48 cm (26th percentile) | OFC 33 cm (10th percentile); weight 3.77 Kg (61st percentile); height 52 cm (76th percentile) | n.a. | weight 2.01 Kg (83rd percentile) | weight 1.11 Kg (3rd percentile) | weight 4.25 Kg (92nd percentile) | OFC 35.5cm (25th percentile); weight 4.11 Kg (87th percentile) | n.a. | Weight 2.35 Kg; height 47 cm | n.a. |
| ***auxological parameters at evaluation (OFC, weight, length)*** | OFC 50 cm (10th centile, -1,31 SD), weight 28 Kg (64th centile, +0,35 SD), height 130 cm (66th centile, +0,40 SD). | OFC 50 cm (3rd-10th percentile), height 134 cm (75th-90th percentile), weight 26 kg (50th -75th percentile) | OFC 53 cm (85th percentile), height 127 cm (46th percentile), weight 22 kg (13th percentile) | n.a. | OFC 52 cm (30th percentile); 34 Kg (28th percentile); height 142 cm (39th percentile) | OFC 47.3 cm (45th percentile); weight 11,4 Kg (30th percentile); height 84 cm (30th percentile) | OFC 53.6 cm (32nd percentile); weight 45 Kg (50th percentile); height 154 cm (30th percentile) | OFC 52.3 cm at 9 years (58th percentile) | OFC on 2nd percentile | OFC <0.4th percentile, weight on 45th percentile; height on 45th percentile | OFC 51.5cm (4th percentile) | OFC 50.2 cm (3rd percentile) at age 21 months | n.a. | OFC 53.5 cm (+0.5 SD), weight 29.5 Kg (-0.75 SD), height 146 cm (+1 SD) | n.a. |
| ***Regression (yes/not, months)*** | not (at 20 months she acquired babbling, but after language development in words did not progress ) | not | not | not | not | not | not (temporary regression of hand use with loss of eating skills) | n.a. | yes; limited to language skills | not | yes; 13 month. At 2 years, loss of sitting position and interest in toys after the onset of epilepsy. | not | n.a. | n.a. | not |
| ***Sitting position (yes/not, age of acquisition)*** | yes; 36 months | yes; 10 months | Yes; 8 months | yes; 10 months | yes; 7 months | yes; 11 months | yes; 7.5 months | n.a. | yes; 2 years | yes; 7 months | yes, 13 months; then regression | not | n.a. | n.a. | yes; 9 months |
| ***Autonomous walking (yes/not, age of acquisition)*** | yes; 21 months, | yes; 18 months | yes; 19 months | not | yes; 23 months | yes; 2 years | yes; 12 months | yes | yes; 4 years and 6 months | yes; 15 months | not | not | n.a. | yes; 17 months | not |
| ***Language (babble speech, first word/ age of acquisition)*** | babble speech (20-30 months) | first words 24 months | babble speech at 12 months, first words at 24 months | 24 months | absence of language | 2 years | yes, delayed, first words at 20 months with stagnation | yes, delayed | babble speech at 5-6 years, first word at 7 years | babble speech at 7 months, first single words at 11 and ½ months | not | not | limited | first word at 12 months, then slow acquisition | babble speech |
| ***Language with phrases (age of acquisition)*** | not | yes, very simple sentences (only two words) | not | not, only single words | not | yes, simple sentences with three words at 4 years | yes, VIQ 106, dyslexia, logopedic aid | yes, impaired (about 40 words at 5 years; few phrases) | not | yes; 16 months | not | not | yes, short sentences at 11 years | yes, she speaks in sentences and counts up to 15 | not |
| ***Gastrointestinal disturbance*** | gastro-esophageal reflux and constipation | gastro-esophageal reflux and constipation | constipation | constipation | constipation | not | reflux; abdominal pain complaints, urge for stool | n.a. | not | not | gastro-esophageal reflux | gastro-esophageal reflux, constipation | n.a. | n.a. | n.a. |

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| ***Cold extremities*** | yes | yes | not | yes | not | not | sometimes cold extremities, difficulties for sensing temperature, problems with cold/heating | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| ***Respiratory disturbance*** | not | hyperventilation and bloating | not | not | not | not | effort-related thoracal complaints, | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| ***Motor disturbance*** | generalized hypotonia, involuntary hands movements, shaking movement involving the whole body | stereotypies of the hands along the midline | hand stereotypies | hand stereotypies from the 5th months | muscular hypotonia; scoliosis and patellar instability | Bayley scales: delay of 10 months; early hypotonia; some hand stereotypies | developmental coordination disorder (fine motor problems); often clumsiness and falls | normal muscle tone and strength; repetitive behaviors | at 10 years, ataxic gait, aggressive and self-injurious behaviour, stereotypies (hand clapping, head shaking, leg kicking, rocking) | poor coordination; she frequently had falls in early childhood | hypotonia | hypotonia | n.a. | stereotypies | repetitive behaviors |
| ***Use of hands*** | good hand use from 12 years old | ability to grasp objects but inability to use objects correctly | reduced and poor hand use | never | reduced use of hands | normal | Yes | n.a. | yes (limited use of cutlery) | yes (learnt to use cutlery and fasten buttons at age 9) | not | not | n.a. | purposeful and stereotypies | poor performance on fine and gross motor |
| ***Severity of intellectual disability*** | moderate | moderate | mild | severe | severe | moderate | IQ average normal :TIQ 100, VIQ 106, PIQ 122, working memory 88, processing speed 83. He is in special education. | Leiter-R scale at 2 years 8 months: IQ/Composite Score of 53 (0.1 centile) | severe | moderate; learning disability | severe | severe | moderate/severe | mild/moderate | severe |
| ***Behaviour*** | autism, hyperactivity. | hyperactivity, self-injurious behavior, autism | autistic behaviour | self-injurious behavior | autism | mood swings; low frustration level | diagnosis of ADHD, autism | autism, ADHD | autistic features and self-injurious behaviour | autism spectrum disorder | n.a. | n.a. | attention deficit/hyperactivity | mild autistic behavior (objects alignments, limited socialization) | autism |
| ***Crisis (onset, type)*** | 19 months, apnea and cyanosis associated with hypertonia and then status epilepticus | 36 months, partial crisis with neck and mandible hypertonia, difficulty in swallowing | n.a. | 12 months | epileptic activity left parietal | only one large seizure event, well responding to Levetiracetam | not; at 3 years, he reported crying spells, anger, aggressiveness, depressive moods | 3.5 years, partial complex seizures; intractable epilepsy with altered consciousness | absence and complex seizures at the age of 3 years | not | 2 years, generalised myoclonic epilepsy | myoclonic type seizures at age 2 years and 6 month. At 13 years tonic-clonic seizures and absence episodes | not | 3 years, absences and falls | 19 months |
| ***EEG*** | electric anomalies in the left temporo-occipital area | paroxysmal focal anomalies | normal | irregular medium voltage theta band background activity symmetrical, poorly reactive. | normal | n.a. | normal | generalized slowing of EEG 4204G>A (p.(Ala1402Thr)); inheritedbackground | epileptiform changes | n.a. | n.a. | in deeper sleep brief runs of rhythmic spikes/sharp waves over centroparietal regions. Flexor spasm followed a generalised irregular slow wave and spike burst rapidly fell asleep again | n.a. | n.a. | background of middle-high voltage sharp wave and sharp slow complex wave with spike slow complex wave in the left temporal region, the left center, and the bilateral occipital region |
| ***Brain MRI*** | slight widening of some hemispherical and cerebellar cortical sulci | slight alteration of the paratrigonal white matter signal, moderate hypodevelopment of corpus callosum | normal | performed around 2 y; mild atrophy of the subarachnoid spaces in bilateral FT,; findings of delayed myelination | normal | normal | 2018: periventricular leukomalacia (right>left), left small periventricular cyst | normal MRI at age 3 and 5 | at 6 years, unmyelinated white matter consistent with an 'immature brain', small degree of volume loss | normal | n.a. | n.a. | n.a. | n.a. | widened ventricle cistern and sulci, patchy hyperintensity in bilateral parietal lobe white matter |
| ***IQSEC2 mutation*** | *de novo* c.4039dup (p.Ala1347Gly*fs**40) | *de novo* c.4110_4111del (p.(Tyr1371Gln*fs**15)) | *de novo* c.3613_3613delC (p.(Leu1205Trp*fs**192)) | *de novo* c.3780delG (p.(Gln1261Serfs*136)) | *de novo* c.4419_4431del (p.(Ser1474Arg*fs**17)) | *de novo* c.3859C>T (p.(Gln1287*)) | c. from mother (reporting normal schooling | *de novo* c.4419delC (p.Ser1474Val*fs*Ter21) | c.4419_4420 insC (p.(Ser1474Gln*fs**133)) (no evidence of skewed X | c.4419_4420 insC (p.(Ser1474Gln*fs**133)) (no evidence of skewed X inactivation, parental mosaicism) | *de novo* c.4419_4420 insC (p.(Ser1474Gln*fs**133)) | *de novo* c.4419_4420 insC (p.(Ser1474Gln*fs**133)) (parental mosaicism) | *de novo* c.4401del (p.(Gly1468Ala*fs**27)) | *de novo* c.4039dup (p.(Ala1347Gly*fs**40)) | *de novo* c.4164dupC: (p.(Ile1389His*fs**218)) |

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| | (NM_001111125.3) | | | | | | with concentration problems and several psychiatric admissions as a child; immune enteropathy and weight problems); inherited from maternal grandmother with concentration problems and dyslexia | | inactivation, parental mosaicism) | | (parentall mosaicism) | | | | |

M = male; F = female; OFC= occipital frontal circumference; n.a. = not available/assessable.

**Table 2. Clinical features of patients harbouring an *IQSEC2* mutation not lying in the C-terminus of the protein**

| Features | Patient 8 | Patient 9 | Patient 10 | Patient 11 | Patient 12 | Family 2 (siblings) | | | Patient 16 |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Patient 13 | Patient 14 | Patient 15 | |
| **Sex** | M | M | F | F | F | M | M | M | M |
| **Age (years)** | 7 | 15 | 7 | 11 | 8 | 34 | 44 | 46 | 20 months |
| **Pregnancy issues** | normal pregnancy | normal pregnancy, C-section, delivery at term | not | placental abruption at 8th week of gestation | normal pregnancy, delayed birth at 40weeks of gestation | normal pregnancy | normal pregnancy | normal pregnancy | maternal cholestasis of pregnancy; PPROM at 24 weeks 6/7 PMA; premature birth at 25 weeks PMA |
| **Auxological parameters at birth (OFC, weight, length)** | OFC 34.5 cm (27th percentile, -0,61SD); weight 3.18 Kg (27th percentile, -0,61SD) | normal somatometry at birth for all parameters | OFC 33 cm (10-25th percentile), weight 3.45 Kg (25-50th percentile); height 50 cm (50th percentile) | OFC 32 cm (10-25th percentile); weight 2.57 Kg (< 10th percentile); height 49 cm (50th percentile) | OFC 33 cm (11th percentile); weight 2.930 Kg (16th percentile); length 48 cm (26th percentile) | normal somatometry at birth for all parameters | normal somatometry at birth for all parameters | normal somatometry at birth for all parameters | OFC 21.5 cm (10th percentile); weight 571 gr (10th percentile); height 30.5 cm (25th percentile) |
| **Auxological parameters at evaluation (OFC, weight, length)** | OFC (at 6 years) 50 cm (13th percentile,-1,13SD); weight 22 Kg (63rd percentile,+0,34SD) | OFC 54 cm (27th percentile, -0.60SD); weight 45.1 Kg (8th percentile, -1.39SD); height 155 cm (15th percentile, -1.06SD) | OFC n.a.; weight 24. Kg (50-75th percentile); height 122 cm (50-75th percentile) | OFC 50 cm (3rd-10th percentile); weight 35 Kg (75th -90th percentile); height 145 cm (90th-97th percentile) | OFC 50 cm (10th percentile); weight 27 Kg (58th percentile); height 128 cm (50th percentile) | OFC 55 cm (47th percentile,-0,07SD); weight 77 Kg (69th percentile, +0,51SD); height 168 cm (12th percentile, -1,2SD) | OFC 54.5 cm (34th percentile,-0,42SD); weight 74.7 Kg (63rd percentile,+0,34SD); height 165 cm (5th percentile,-1,62SD) | OFC 53.4 cm (12th percentile,-1,16SD); weight 78 K g (71st percentile ,+0,55SD); height 172 cm (26th percentile ,-0,64SD) | OFC 46 cm (6th percentile); weight 8.3 Kg (<1st percentile, -3.2SD); height 74 cm (<1st percentile, -2.9SD) |
| **Regression (yes/not, months)** | not, but motor and developmental delay from 6 months | not; always positive evolution | not | yes, late regression; after five years, she lost the ability to hold objects, after 9 years she lost motor skills | not | not | not | not | not |
| **Sitting position (yes/not, age of acquisition)** | not | yes; 10 months | yes; 7 months | yes; 12 months | yes; 16 months | yes; 1 year | yes; 1 year | yes; 9 months | yes; 8 months |
| **Autonomous walking (yes/not, age of acquisition)** | not | yes; 24 months | yes; 20 months | yes; 7 years. Anyway, Unstable walking | yes, 19 months; then, a pause between 19-22 months. After, walking on tips | yes, 2.5 years; slow and clumsy walking in adult age | yes, 3 years; slow and clumsy walking in adult age | yes, 14 months; slow walking in adult age | yes 20 months |
| **Language (babble speech, first word/ age of acquisition)** | absence of language | first word at 18 months | first words at 15 months | absent | absence of language | babble speech at 7 months; first word at 3 years. He currently speaks, repeats words | babble speech at 8 months; first word at 2.5 years. He currently speaks, repeats words a | babble speech at 4 months; first word at 1 years. He currently speaks, but not clearly | yes; 5 months |

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| | | | | | | a lot and sometimes is not understood | lot and sometimes is not understood | | |
| **Language with phrases (age of acquisition)** | not | 3 years. Nowadays: few words, simple sentences (understands french, catalan and spanish) | not | not | absence of language | 7 years | 8 years | 3 years | use of three single words at 20 month no phrases yet |
| **Gastrointestinal disturbance** | constipation; swallowing problems; feeding by NGT | not; acquired sphincter control | not | constipation | constipation | n.a. | n.a. | during infancy, two or three episodes of diarrhea | not |
| **Cold extremities** | not | not | not | yes | not | not | not | not | n.a. |
| **Respiratory disturbance** | not | not | not | episodes of apnea | not | n.a. | n.a. | n.a. | neonatal respiratory distress due to premature birth; no any issues at 8 months |
| **Motor disturbance** | severe hypotonia; GMFCS level V stereotypies | waving stereotype | hand stereotypies | hypotonia and poor head control , manual stereotypies from the age of 15 months.(hand washing, hand mouthing and tongue protruding movements) | scoliosis; walking on tips; hands stereotypies Rett-like | problems with fine motor movements; when walking, he has a broad base of support | problems with fine motor movements; when walking, he has a broad base of support | problems with fine motor movements | not |
| **Use of hands** | absent | acquired | yes | yes, grabbing objects from the age of 13 months until 4-5 years. Then, regression. | she can use spoons, but not pencils | problems with fine motor movements | problems with fine motor movements | problems with fine motor movements | normal use of hands at 20 months |
| **Severity of intellectual disability** | severe- profound | mild- moderate | severe | severe | severe | moderate | moderate | moderate | Alberta Infant Motor scale at 8 months: score 17/25 (25th percentile); Bayley-III at 20 months within normal range (score 85-16th percentile) |
| **Behavior** | restless, ASD | emotional lability, social difficulties but he participates in conversation with strangers, explores. Mild ASD traits. | autistic behaviour | self-injurious behavior, autism | autism, aggressive behavior; immotivate smiling, abnormal sleep | occasional aggressiveness and irritability | occasional aggressiveness and irritability | occasional aggressiveness and irritability | normal |
| **Crisis (onset, type)** | 3 years, generalized tonic, focal clonic, absence | absent | 24 months, absence episodes | 18 months, gaze fixity, localized tremors and sialorrhea and tonic-clonic crises | not | 6 months, generalized, with fever | 6 months, generalized, with fever. Currently, epileptic seizures difficult to control | 18 months, generalized, with dehydration and diarrhea | not |
| **EEG** | frequent multifocal epileptiform discharges | not performed, never seizure suspicion nor febrile crisis | n.a. | slowing of the basic rhythm, slowing of the rhythm in the left hemisphere, | normal | abnormal during infancy; slow activity in adult age | abnormal during infancy and adult age; currently, epileptic activity | abnormal during infancy; slow activity in adult age | EEG at 29 weeks of gestation: normal background EEG with prominent delta |

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| | | | | paroxysmal activity in the left front-temporal and right frontal region | | | | | brushes, no burst suppression. Multifocal epileptic activity (mostly from a central-right focus and bilateral occipital) |
| **Brain MRI** | normal | MRI performed in other structures; reported slightly increased CSF spaces in the frontal region | alteration of the white matter signal | subarachnoid spaces ectasia, thinning of the corpus callosum | normal | unrealized | unrealized | unrealized | abnormal corpus callosum with aplasia of the anterior part of the truncus; right-sided hemorrhagic periventricular focus at the interface of the capsula externa and the putamen |
| **IQSEC2 mutation** | *de novo*, c.1881delC (p.(His629Metfs*4)) | *de novo*, c.1591C>T (p.(Arg531*)) | *de novo* c. 267C>G (p.(Thr89*)) | *de novo* c.3011T>C (p.(Leu1004Pro)) | *de novo*, c.2911C>T (p.(Arg971*)) | c.1076G>A (p.(Arg359His)) | c.1076G>A (p.(Arg359His)) | c.1076G>A (p.(Arg359His)) | c.999+8A>G; maternally inherited |

M = male; F = female; OFC= occipital frontal circumference; n.a. = not available/assessable.

**Table 3. Clinical features of patients harbouring a CNV encompassing the *IQSEC2 gene***

| ***Features*** | **Patient 17** | **Patient 18** | **Family 3** | | |
|---|---|---|---|---|---|
| | | | **Patient 19 Son** | **Patient 20 Daughter** | **Patient 21 Mother** |
| ***Sex*** | M | F | M | F | F |
| ***Age (years)*** | 7 | 19 | 14 | 19 | 45 |
| ***Pregnancy issues*** | normal pregnancy | normal pregnancy, C-section, delivery at term | normal pregnancy | normal pregnancy | normal pregnancy, delayed birth at 40weeks of gestation |
| ***Auxological parameters at birth (OFC, weight, length)*** | OFC 33 cm (10th percentile, -1,27SD); weight 3.28 Kg (33rd percentile, -0,45SD); height 51.5 cm (69th percentile, +0,51SD) | normal OFC and height; weight 2.7 Kg (8th percentile, -1.38SD) | OFC 33.5 cm (15th percentile); weight 3.45 Kg (43rd percentile); height 51 cm (62nd percentile) | OFC 32.5 cm (6th percentile);weight 4.30 Kg (97th percentile); height 51 cm (71st percentile) | n.a |
| ***Auxological parameters at evaluation (OFC, weight, length)*** | OFC (at 5 years) 50 cm (24th percentile, -0,62SD); weight 30 Kg (>99th percentile, +3,69SD) | OFC 52 cm (39th percentile, -0.28SD) | OFC 51 cm (1st percentile, -2.3SD); weight 47 Kg (30th percentile); height 178 cm (97th percentile) | OFC 55 cm (73rd percentile); weight 62 Kg (61st percentile); height 165 cm (61st percentile) | n.a. |
| ***Regression (yes/not, months)*** | not | yes, regression of the use of hands at 2 years | not, but very slow development | not | not |
| ***Sitting position (yes/not, age of acquisition)*** | yes; 30 months | yes; 14 months | yes; 21 months | yes, 9 months | n.a. |
| ***Autonomous walking (yes/not, age of acquisition)*** | not | yes; 2 years | yes, 24 months | yes; 14 months | n.a. |
| ***Language (babble speech, first word/ age of acquisition)*** | absence of language | babble speech a t 1 years | absence of language | 2 years | n.a. |
| ***Language with phrases (age of acquisition)*** | never | never | absence of language | 3.5 years | normal language skills |
| ***Gastrointestinal disturbance*** | constipation; lactose intolerance | not; acquired sphincter control | not | not | not |
| ***Cold extremities*** | not | small and cold feet | not | not | not |
| ***Respiratory disturbance*** | not | not | not | not | not |
| ***Motor disturbance*** | hypotonia; not autonomous walking; walking on knees; stereotypies | washing stereotype, hands to face since 17 months | hypotonia, problems in walking and in maintaining the balance; repetitive hands movements | clumsiness and poor motor coordination | clumsiness and poor motor coordination |
| ***Use of hands*** | absent | acquired at 1 year, lost at 2years; at 9years, she eats alone with fork and spoon and takes toys | he can eat | acquired with no problems | acquired with no problems |
| ***Severity of intellectual disability*** | severe- profound | moderate | severe intellectual disability | moderate intellectual disability | moderate intellectual disability |
| ***Behavior*** | restless, ASD | behavioral disturbances, ASD, teeth grinding | autism-like and auto/ etero aggressiveness; he lives in a protect institution | autism- like; she lives in a protect institution | autism- like; she lives in a protect home |
| ***Crisis (onset, type)*** | 5 years, generalized tonic clonic | 9 years, generalized | 9 years, tonic- clonic | not | not |
| ***EEG*** | normal | generalized discharges activated in sleep; slow background | Epileptic activity left frontal | not | not |

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| ***Brain MRI*** | periventricular and frontal delayed myelination | normal | normal | not performed | not performed |
| ***IQSEC2 mutation*** | *de novo*, partial duplication of 0,25 Mb ChX(GRCh37):g.53270956-53296256 | *de novo*, deletion of 0,4 Mb ChX(GRCh37):g.52954520-53394275 | inherited, partial duplication of 0,41 Mb ChX(GRCh37):g53300438-53341868 | inherited, partial duplication of 0,41 Mb ChX(GRCh37):g53300438-53341868 | partial duplication of 0,41 Mb ChX(GRCh37):g53300438-53341868 |
| | | | additional inherited duplication of 0,55 Mb ChX(GRCh37):g.139888816-140443674 | | additional duplication of 0,55 Mb ChX(GRCh37):g.139888816-140443674 |

M = male; F = female; OFC= occipital frontal circumference; n.a. = not available/assessable.

**Fig1. IQSEC2 protein domain and mutation schematic.** Schematic diagram of the protein domain structure of IQSEC2 (N-terminal coiled coil (CC) domain, IQ calmodulin-binding motif (IQ), SEC7 and Pleckstrin homology (PH) domains, and PDZ-binding motif (STVV)) with amino acid numbers provided. Mutations previously reported in other studies are shown in green lines (above), the mutations identified in the patients described in the present study are indicated in red (below), chromosomal regions involved in CNVs are highlighted with a red dashed line.