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RESEARCH NOTE

Psychiatric features and variable neurodevelopment outcome in four females with IQSEC2 spectrum disorder

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Abstract. *IQSEC2* is an X-linked gene highly expressed at the excitatory synapses where it plays a crucial role in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor trafficking and synaptic plasticity. To date, several males and females with severe to profound intellectual disability have been reported harbouring frameshift and nonsense variants in this gene, whereas a milder phenotype has been recognized in females carrying missense pathogenic variants. Here, we report two novel *IQSEC2* variants in four females with psychiatric features and otherwise variable cognitive impairment. A female (case 1) with severe verbal language learning disorder and a psychotic episode (precipitated by exposure to anti-contraceptive pill) harboured a *de novo* pathogenic frameshift variant (c.1170dupG,p.Gln391Alafs*5), whereas the female proband of family 2, displaying severe psychomotor regression and complex psychiatric features carried a missense variant of uncertain significance (c.770G>A,p.Ser257Asn) that was maternally inherited. Skewed X-inactivation was noted in the carrier mother. The maternal aunt, affected by schizophrenia, was found to bear the same *IQSEC2* variant. We discuss the variable clinical presentation of IQSEC2 spectrum disorders and the challenging genotype–phenotype correlation, including the possible role of environmental factors as triggers for decompensation. Our report highlights how psychiatric features may be the main clinical presentation in subtle IQSEC2 phenotype, suggesting that the prevalence of IQSEC2 mutations in patients with psychiatric disorders may be underestimated.

Keywords. *IQSEC2* gene; psychiatric disorders; intellectual disability; postsynaptic density; small GTPases.

Introduction

Neurodevelopmental disorders are clinically and genetically heterogeneous group of diseases with overlapping pathophysiology, involving neurogenesis, cell migration and neuronal connectivity (Waltereit *et al*. 2014; Shohat *et al*. 2017). A continuously expanding number of presynaptic and postsynaptic genes have been associated with various neurodevelopmental disorders, including intellectual disability (ID), autism spectrum disorder (ASD), schizophrenia and epilepsy (Taoufik *et al*. 2018).

IQSEC2, located at Xp11.22, is highly expressed at the postsynaptic density (PSD) of excitatory synapses, where it plays a crucial role in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) trafficking and synaptic plasticity (Brown *et al*. 2016; Petersen *et al*.

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2018, 2020). The *IQSEC2* gene encodes the IQ motif and Sec7 domain-containing protein 2 which has a few functional domains: the N-terminal coiled coil (CC) domain; the IQ calmodulin-binding motif (IQ) which is thought to accelerate the guanine nucleotide exchange factor (GEF) activity; the Sec7 domain that bears GEF activity catalyzing the switch from guanosine diphosphate (GDP) to guanosine triphosphate (GTP) on target Arf proteins; the Pleckstrin homology (PH) domain which can mediate membrane localization, and the PDZ-binding motif (STVV) which binds the protein to the PSD of excitatory synapses.

During the last decade, several IQSEC2 pathogenic variants have been largely reported in both nonsyndromic (Shoubridge *et al*. 2010) and syndromic cases (Tran Mau-Them *et al*. 2014; Alexander-Bloch *et al*. 2016; Helm *et al*. 2017), making IQSEC2 one of the most common causes of X-linked ID. Additional features may include ASD (Zipper *et al*. 2017), epilepsy (Zerem *et al*. 2016; Mignot *et al*. 2019), psychomotor regression and psychiatric features (Alexander-Bloch *et al*. 2016; Radley *et al*. 2019). However, patients with psychiatric symptoms who do not have ASD or ID are not routinely screened for changes in *IQSEC2,* so there is no information about the prevalence of IQSEC2 spectrum disorder in such patients. What is known is that both males and females could be affected, usually presenting with severe to profound ID when they carry *de novo* nonsense and frameshift variants (Tran Mau-Them *et al*. 2014; Ewans *et al*. 2017; Barrie *et al*. 2019). In contrast, mild to moderate ID (Zerem *et al*. 2016; Mignot *et al*. 2019) and occasionally normal intelligence (Shoubridge *et al*. 2010; Mignot *et al*. 2019) have been reported in females harbouring inherited missense *IQSEC2* variants. The presence of language regression, stereotypic hand movements, and microcephaly in some females has pointed to consider *IQSEC2* in the spectrum of Rett-like syndromes (Tran Mau-Them *et al*. 2014; Alexander-Bloch *et al*. 2016; Allou *et al*. 2017; Radley *et al*. 2019). *IQSEC2* pathogenic variants have also been suggested to cause overlapping phenotype with Smith–Magenis syndrome (Berger *et al*. 2017) and Angelman syndrome (Radley *et al*. 2019), two further neurodevelopmental disorders presenting with behavioural problems.

Here, we report four females with genetic changes in *IQSEC2*. We highlight how psychiatric features may be the main clinical presentation in subtle IQSEC2 phenotype and emphasize the possible attenuating and aggravating factors that may modulate the phenotype, based on our experience.

Material and methods

Basic work-up for ID/ASD

Extensive metabolic work-up, comparative genomic hybridization (CGH)-array and Fragile-X testing were pursued in both cases 1 (III-2) and 2 (III-1) (figure 1, a&f), to rule out relevant metabolic genetic conditions, pathogenic copy number variants or CGG expansion in the *FMR1* gene, respectively. All these screening tests are part of the standard work-up for patients with neurodevelopmental diseases (Michelson *et al*. 2011; van Karnebeek *et al*. 2014) and yielded negative results.

Gene panel sequencing

As a further step, a genetic panel including about 2500 genes targeting disorders associated with ID/ASD (GeneDx) was performed in both cases 1 (III-2) and 2 (III-1). Given the complexity of clinical presentation, case 2 (III-1) also underwent an extended mitochondrial genome and nuclear panel with more than 1400 genes.

Whole-exome sequencing

Further, to investigate other possible genes not included in the above panels, analysis of whole-exome sequencing (WES) data was performed in cases 1, 2 and 3 after appropriate consent was obtained. Using genomic DNA from the submitted specimen(s), the exonic regions and flanking splice junctions of the genome were sequenced by massively parallel (NextGen) sequencing on an Illumina sequencing system with 100 bp at GeneDx (Gaithersburg, USA). Exon-level read counts were determined using GATK v4 depth of coverage. Removal of duplicate reads, mean coverage of coding sequence regions, alignment and variant annotation were performed using analytical pipelines that include publicly available tools and custom scripts at the Research Institute of the McGill University Health Centre (Montreal, Canada). We looked at nonsynonymous exonic and splicing variants with a minor allele frequency ≤ 0.001 in gnomAD (Genome Aggregation Database) database.

X-chromosome inactivation study

Lastly, to further assess a possible different pattern of X-chromosome inactivation among cases 2 (III-1), 3 (II-2) X-inactivation study was performed as previously determined through DNA digestion with restriction endonucleases sensitive to cytosine methylation (*Hpa*II) and polymerase chain reaction (PCR) amplification of CAG (cytosine–adenine–guanine) repeat in androgen receptor gene (Allen *et al*. 1992).

Results

Clinical report

Case 1: The proband (III-2) was the first child to healthy nonconsanguineous parents of Philippines ancestry

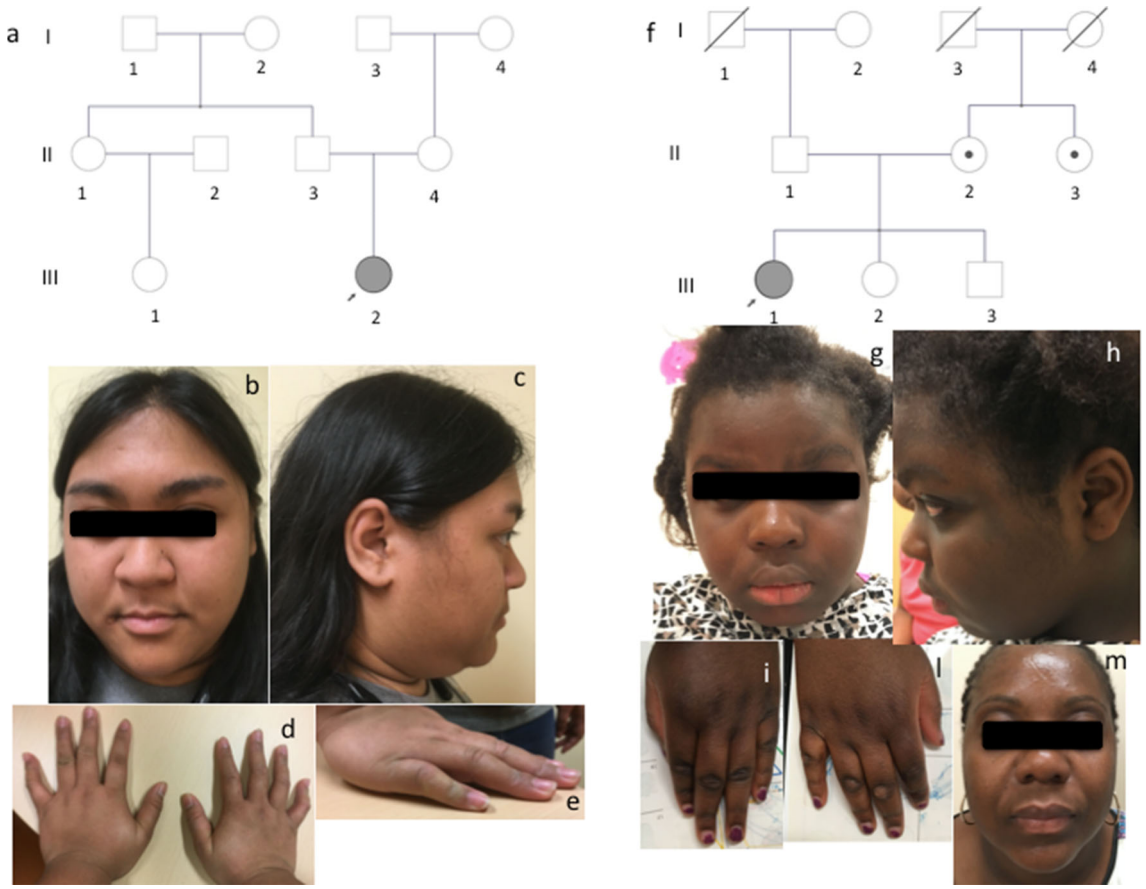

Figure 1. (a) Pedigree and photos of case 1, case 2 and her mother carrying IQSEC2 variants. Pedigree showing the affected cases 1 and 2 (III2 panel (a) and III1 panel (f), respectively) in the two families indicated by an arrow that points to a full grey circle. Mother (II2) and aunt (II3) of case 2 (III1) carrying the same missense variant c.770G>A,p.Ser257Asn are indicated with a dot. (b) Case 1 harbouring c.1170dupG,p.Gln391Alafs*5 variant showed broad eyebrows, upslanting palpebral fissures, broad nasal bridge and nasal base, mild everted lower lip and pronounced chin (c), large ear lobe, squared palm of hands, camptodactyly of V finger bilaterally and tapering fingers (d, e). Case 2 carrying the c.770G>A,p.Ser257Asn variant, showed short nose, thick ale nasi, broad nasal base, (g) full lips, (h) thick earlobe, (i–j) distally tapered fingers with marks of self-injury behaviours (m). Her mother showed high forehead, broad nasal base with thick ale nasi, full lips but overall no coarse features as her daughter.

(figure 1a). Family history was unremarkable except for developmental delay in one paternal cousin in the context of difficult delivery. The proband was born at term, after an uneventful pregnancy, by C-section because of cord coiling. There were no perinatal complications. She acquired gross and fine motor milestones at the expected age. Speech delay was noticed during childhood and eventually she attended a special class due to learning disabilities. Her past medical history was remarkable for obesity since adolescence and polycystic ovarian syndrome (PCOS), treated with metformin. She came to our attention at the age of 16 when she was admitted to the hospital for new-onset acute psychosis, shortly after the initiation of an oral-contraceptive pill (OCP), for secondary amenorrhea. More specifically after one day of exposure to Ariane, an OCP which consists of cyptoterone acetate and ethinylestradiol, the patient started to experience slowness in her daily tasks, deficits in executive function, visual and auditory hallucinations and disorganized thoughts, followed by intermittent headache and brief self-resolving staring episodes. Her mother promptly stopped the OCP medication as she thought it caused the new-onset psychosis, which had never been experienced before. During the admission, she was only able to respond to our questions with very brief answers. Moreover, improper laughing episodes and abnormal repetitive behaviour were noticed. Dysmorphological evaluation showed broad eyebrows, upslanting palpebral fissures, broad nasal bridge and nasal base, mild everted lower lip and pronounced chin, anterior open bite, large ear lobes, squared palm of hands, camptodactyly of V finger bilaterally and tapering fingers (figure 1, b–e). She had acanthosis nigricans over her neck. She was promptly started on abilify with progressive resolution of the acute psychosis. Brain MRI, EEG and ophthalmological evaluation resulted normal. There were no focal deficits on neurological assessment. Growth parameters were normal: height 163 cm (50th percentile), weight 94.4 kg (>90th percentile), and occipital circumference (OFC) 56 cm (50th percentile). A full neuropsychological

assessment conducted at the age of 19, revealed significant deficits in both receptive and expressive language, whereas the patient's nonverbal abilities were in the lowest end of the average range. This severe language disability had significant impact on daily life functions.

Cases 2,3,4: The proband of family 2 (case 2, III-1) was the first of three siblings who were healthy and had normal development. Her parents were originally from Congo and overall healthy. Her mother (case 3, II-2) reported that she experienced an episode of depression with psychotic features, right after her third pregnancy, in the context of significant stress and poor sleep. She was started on olanzapine, which was replaced by aripiprazole due to weight gain, and promptly recovered a few weeks later. She denied learning difficulties or further psychiatric issues. Family history was also remarkable for a maternal aunt (mother's sister, case 4, II-3) with schizophrenia (figure 1f).

The proband was born full term after an uneventful pregnancy. There were no perinatal complications. She acquired early developmental milestones at the expected age. Yet, parents started to have concerns about her development around the age of 3 years, when they noticed language delay. Since then she started to experience progressive psychomotor regression associated with several behaviour issues. A diagnosis of primary psychotic disorder (schizophrenia versus schizoaffective disorder) was established during early childhood. She was promptly started on multiple antipsychotic medications without a significant improvement. She partially responded only to aripiprazole. At the age of 8 years she was diagnosed with precocious puberty. Brain MRI revealed enlarged pituitary with two focal lesions suggestive of microadenoma. A full neuropsychological assessment conducted at the age of 9 years, revealed severe impairment of executive functions with sensorimotor and visuo-spatial deficits and attention deficiency, albeit a cognitive profile in the low normal range. Over the last 4 years, she progressively lost most of the previously acquired developmental milestones, and at the time of most recent evaluation when the patient was 13 years old, she was not able to talk nor follow simple commands, run, draw, or use utensils to eat. She exhibited disorganized thought and speech with visual hallucinations, disinhibited behaviours, recurrent episodes of agitation and aggression. She was agitated and it was impossible to perform a full physical exam. No focal deficits were obvious on neurological exam. OFC was 61.5 cm (about +4.9 SD), in the context of relative familial macrocephaly (her father had 59 cm, +2.7 SD). The remaining physical examination revealed coarse features including short nose, thick ale nasi, broad nasal base and full lips (figure 1, g–l).

The mother (case 3, II-2) was healthy at the time of the genetic evaluation. She reported history of a brief psychotic episode which took place after the delivery of her affected daughter. At the time of our evaluation, her neurological examination was normal and no specific dysmorphisms were noted (figure 1m).

The 29 year-old aunt (case 4, II-3) was known for a diagnosis of schizophrenia since the age of 20. Her development and cognitive profile were grossly normal. Her medical history was remarkable for transient episodes of neck dystonia in her early 20s and short term memory loss since adolescence. Her neurological examination was normal, except for synkinesia. There were no dysmorphisms.

Genetic investigations

The ID panel revealed a *novel, de novo* frameshift variant (c.1170dupG,p.Gln391Alafs*5) in *IQSEC2* (NM_001111125.1) in case 1 (III-2). The new reading frame ends in a stop codon 5 positions downstream, likely resulting in a degraded protein. According to the American College of Medical Genetics (ACMG) guidelines this variant can be classified as pathogenic, bearing the PVS1 (null variant in a gene where loss-of-function is a known mechanism of disease), PS1 (*de novo*), PM2 (absent from controls, including gnomAD) (Richards *et al*. 2015).

A maternally inherited variant of unknown significance (VUS) (c.770G>A,p.Ser257Asn) in *IQSEC2* (NM_001111125.1) was identified in case 2 (III-1) by the ID panel. This variant has been reported only once in gnomAD (ALL 1/134043). The maternal aunt (case 4, II-3) was found to harbour the same *IQSEC2* variant. The mitochondrial genome and nuclear panel in case 2 (III-1) resulted normal.

WES analysis did not reveal any pathogenic or likely pathogenic variant in other OMIM genes in cases 1, 2, 3 who underwent exome-sequencing. All patterns of inheritance were considered. A list of the rare variants identified is available at electronic supplementary material at http://www.ias.ac.in/jgenet/. X-inactivation study in blood showed skewed X-inactivation (SXI) (ratio 80%) in the mother and random X-inactivation in the proband. We were not able to perform X-inactivation study on the aunt.

Discussion

Since the first report, a decade ago, of a female patient with ID and epileptic encephalopathy carrying a chromosomal translocation t(X; 20) (p11.2;q11.2) that disrupted *IQSEC2*, roughly 80 patients harbouring *IQSEC2* pathogenic variants have been described (Zerem *et al*. 2016; Kalscheuer *et al*. 2016; Mignot *et al*. 2019; Radley *et al*. 2019; Barrie *et al*. 2019; Rogers *et al*. 2019). The genotypic spectrum of pathogenic variants has recently been reviewed (Shoubridge *et al*. 2019), and a summary can be found in figure 2. With the exception of a few females carrying missense variants (Shoubridge *et al*. 2010; Mignot *et al*. 2019), most affected individuals have moderate to profound ID with or without dysmorphic features (Shoubridge *et al*. 2010; Alexander-Bloch *et al*. 2016; Helm *et al*. 2017). As seen in our cases, the dysmorphic features are typically not

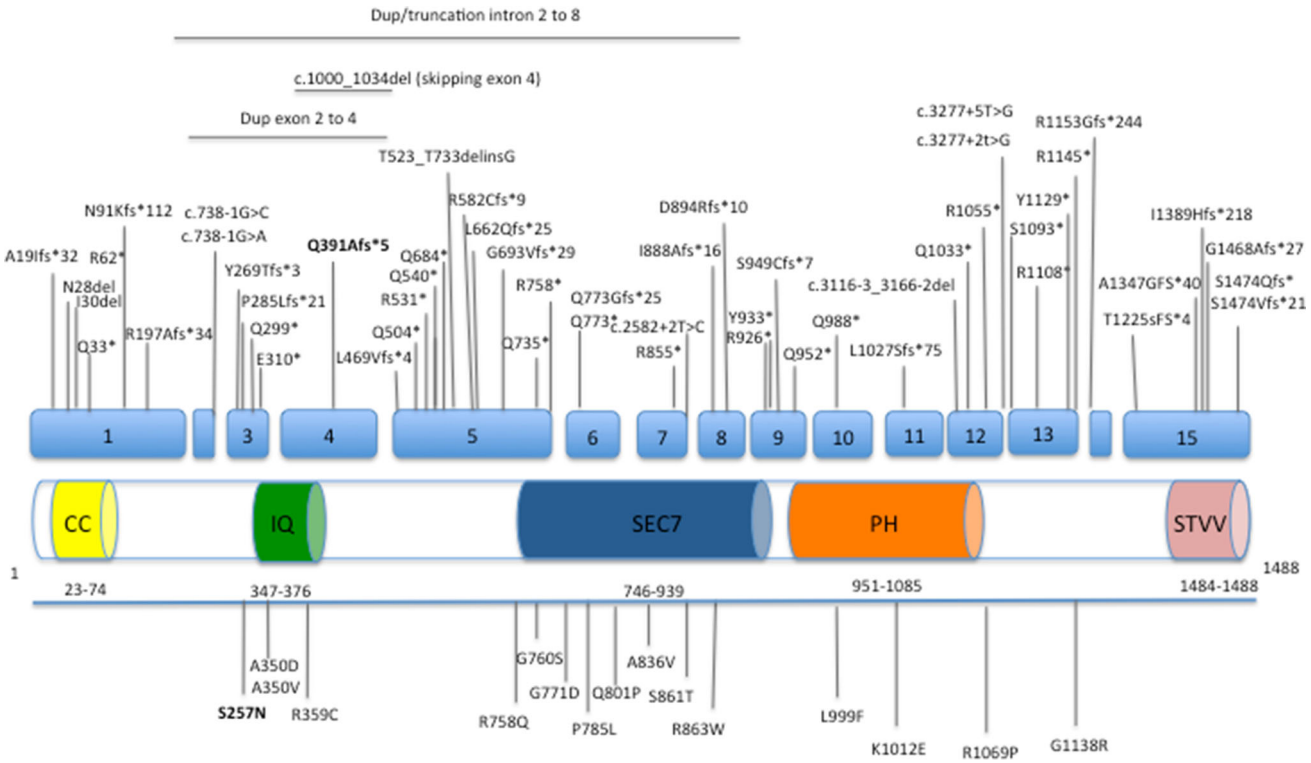

Figure 2. Schematic representation of pathogenic variants along the entire *IQSEC2* gene. Nonsense, frameshift, splicing, indel IQSEC2 variants and missense variants previously reported in the literature are displayed (above and below, respectively) on the schematic representation of the longest NM_001111125.2 isoform (numbered blue boxes correspond to exons) and corresponding protein (NP_001104595) domains: N-terminal coiled coil (CC) domain, IQ calmodulin-binding motif (IQ), SEC7 and Pleckstrin homology (PH) domains, and PDZ-binding motif (STVV). The variant p. Q391Afs*5 carried by case 1 and the variant p. S257N carried by cases 2, 3, 4 are displayed in bold.

specific, suggesting that physical examination is not specific-enough to suggest the diagnosis of IQSEC2 disease (Tran Mau-Them *et al*. 2014; Alexander-Bloch *et al*. 2016; Helm *et al*. 2017). As recently reviewed (Zerem *et al*. 2016), up to 40% of IQSEC2 patients develop epilepsy, displaying a broad range of seizure types from infantile spasms to epileptic encephalopathy. Of note, the recent report of psychomotor regression in several IQSEC2 cases without epilepsy has suggested that it is a distinct feature of IQSEC2-related disorders rather than merely a consequence of epilepsy (Radley *et al*. 2019). This finding is supported by our case 2 (III-1) presenting with psychomotor regression in the absence of epilepsy. Of note, psychomotor regression, psychiatric features and several behavioural issues have been largely reported (Tran Mau-Them *et al*. 2014; Alexander-Bloch *et al*. 2016; Zerem *et al*. 2016; Radley *et al*. 2019), although little is known about prevalence, age of onset and course of psychiatric diseases among IQSEC2-related disorders. Remarkably, besides ASD, nonspecific psychiatric features have been previously reported in a boy and his mother carrying the missense variant p.Arg863Trp (Shoubridge *et al*. 2010). Of interest, all cases reported in our manuscript had psychiatric symptoms, and case 2 (III-1), in particular, had an unusually early diagnosis of psychotic disorder.

A genotype–phenotype correlation has recently been reported, suggesting that *de novo* nonsense and frameshift variants are responsible for syndromic and severe ID, whereas inherited missense variants result in a milder non-syndromic phenotype, especially in females (Mignot *et al*. 2019). In contrast to the majority of previous reports, case 1 (III-2) in our manuscript, carrying the *de novo* frameshift variant c.1170dupG,p.Gln391Alafs*5, classified as pathogenic according to the ACMG guidelines, showed severe language disability but preserved global cognition. The diagnosis was uncovered only after she experienced an episode of psychosis possibly triggered by OCP intake. According to the 50-nucleotide rule (Chang *et al*. 2007), we expect that the presence of c.1170dupG,p.Gln391Alafs*5 leads to nonsense-mediated mRNA decay for the product of that allele.

Moreover, case 2 (III-1) harboring the maternally inherited missense variant c.770G>A,p.Ser257Asn showed severe psychomotor regression with complex psychiatric features, following early normal development.

The c.770G is highly conserved across species (GERP score 5.0599) and its change c.770G>A,p.Ser257Asn is predicted to have a deleterious effect according to various predictive tools (based on SIFT, DANN, CADD). It lies between the N-terminal coiled coil domain and IQ calcium-

binding motif. Although IQSEC2 pathogenic variants have been identified throughout the entire gene, variants affecting the IQ motif have been the most functionally studied and they have been shown to impair IQSEC2 GEF activity. Unfortunately, we were not able to pursue functional studies to confirm the mechanism of the *IQSEC2* changes reported. As mentioned above, IQSEC2's GEF activity is required for activity-dependent trafficking of AMPARs and IQSEC2 is also able to bidirectionally regulate synaptic transmission in a manner independent of this activity (Petersen *et al*. 2020). Overall, a wide range of neurodevelopmental disorders ranging from ID and ASD to various psychiatric diseases have been linked to pathogenic variants in genes involved in GTPases homeostasis (Reichova *et al*. 2018; Zamboni *et al*. 2018). Functional studies in related animal models have revealed dysfunction of dendrites, axons and synapses, suggesting common underlying pathomechanism via abnormal small GTPase signalling (Zamboni *et al*. 2018). Consequently, we hypothesize that imbalance between excitatory and inhibitory synaptic activity may also occur in patients with IQSEC2 mutations, resulting in PSD dysfunction and in turn leading to neuropsychiatric diseases such as schizophrenia and ASD (de Bartolomeis *et al*. 2014; Gao and Penzes 2015). In conclusion, based on our literature review, we hypothesize that a possible deleterious effect of this variant may be mediated by impairment of the small GTPase signalling but other yet unknown mechanisms are also possible, like protein–protein interactions and regulatory effects of AMPAR trafficking (Petersen *et al*. 2020). Further IQSEC2 cases with variants in this protein region are needed to shed light on the possible underlying pathomechanism.

The lack of other pathogenic variants in OMIM genes during the analysis of the WES data supports that the variants identified in *IQSEC2* gene may, indeed, be the explanation for the clinical presentation of the individuals described in this case report. Having said this, extremely variable clinical presentation has been reported among individuals carrying the same IQSEC2 mutation and even twin phenotype discordance has been observed, suggesting that other genetic factors, like imprinting or X-inactivation, and environmental contributors may act as possible phenotype modulators (Radley *et al*. 2019). In our case 2 (III-1), although psychomotor regression is part of the phenotypic spectrum of IQSEC2 disorders, the normal early development and the significantly different presentation from her mother suggest a possible second genetic or environmental hit in case 2 (III-1), dramatically resulting in a severe phenotype. However, despite extensive work-up no other genetic hit was identified. Sex hormones are thought to play a major role in psychiatric disorders (Yazici *et al*. 2013; Platt *et al*. 2017). Given the precocious puberty present in this patient, in the context of a possible microadenoma, related hormonal changes might have been the second hit that precipitated the neuropsychiatric changes of patient 2 (III-1). Of note, precocious puberty has already been reported in an IQSEC2 case (Radley *et al*. 2019). Similarly, hormonal changes due to exposure to OCP may have contributed to the precipitation of symptoms in case 1 (III-2), while the hormonal changes associated with pregnancy and delivery may have acted as a trigger that precipitated a psychotic episode in the mother (II-2) of case 2. Interestingly, random X-inactivation was observed in patient 2 (III-1) and skewed X-chromosome inactivation (SXI) in her mother. IQSEC2 escapes inactivation in humans (Carrel and Willard 2005) but SXI has been reported in a few IQSEC2 females (Zerem *et al*. 2016; Mignot *et al*. 2019). One can thus not rule out the possibility that in this case preferential inactivation of the mutant X chromosome may have led to a milder phenotype in the case of the mother (II-2) of patient 2, thus contributing to the variable clinical presentation in this family. Having said this, the psychiatric features in the cases presented are likely best explained using a multifactorial inheritance model, involving multiple susceptibility genes and environmental factors, with the variation in IQSEC2 being just one of the contributing susceptibility factors (Uher and Zwicker 2017; Campbell *et al*. 2018).

The lack of details about psychiatric presentation, psychomotor regression, possible triggers and response to psychiatric medications in previous IQSEC2 cases should encourage a retrospective and prospective characterization of patients with IQSEC2 spectrum disorders having these elements in mind. The development of an open registry for this information would permit data pooling of greater numbers of rare conditions, which could help identify triggers exacerbating patients' condition, similar to what is available for porphyria (http://www.drugs-porphyria.org) and thus potentially help in the management of individuals with IQSEC2 variants. For example, identifying environmental contributors modulating outcome in these patient (such as sex hormones that seem to be important in all three mutation carriers in our manuscript: OCP, pregnancy related hormonal changes, precocious puberty) could potentially help us to prevent transient psychiatric episodes (as seen in case 1 (III-2) and mother (II-2) of case 2 or even psychomotor regression (patient 2 (III-1). Moreover, such a registry would facilitate research on potentially new drug targets by studying cell lines from families interested in research initiatives.

In conclusion, we have reported two novel IQSEC2 variants in four females with variable clinical presentation. Although the role of one of the variants reported, i.e. the missense variant c.770G>A,p.Ser257Asn remains controversial, our study raises some important points. First, the genotype–phenotype correlation previously reported may not be generalizable, since case 1 (III-2) with a frameshift variant and case 2 (III-1) with a missense variant, displayed mild and severe phenotype, respectively. Secondly, environmental contributors may modulate outcome and psychiatric features may be the only presentation when deficits are subtle making the genetic diagnosis very challenging. As future directions, we recommend the creation of an open registry for carriers of variants in

IQSEC2 to best address these questions. We also recommend screening a large psychiatric cohort for variants in *IQSEC2* to estimate the prevalence of carriers in patients followed in psychiatric clinics where our patients were referred from. We believe our suggestions will help in improving the characterization, as well as the management, of patients with this challenging diagnosis.

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