Received: 26 July 2018 | Revised: 11 October 2018 | Accepted: 15 October 2018

DOI: 10.1002/humu.23670

MUTATION UPDATE

WILEY HGVS HUMAN GENOME VARIATION SOCIETY

IQSEC2 mutation update and review of the female-specific phenotype spectrum including intellectual disability and epilepsy

Cheryl Shoubridge[1,2] | **Robert J. Harvey**[3,4] | **Tracy Dudding-Byth**[5,6]

[1]Department of Paediatrics, University of Adelaide, Adelaide, South Australia 5005, Australia

[2]Robinson Research Institute, University of Adelaide, Adelaide, South Australia 5005, Australia

[3]School of Health and Sport Sciences, University of the Sunshine Coast, Maroochydore DC, Queensland 4558, Australia

[4]Sunshine Coast Health Institute, Birtinya, Queensland 4575, Australia

[5]NSW Genetics of Learning Disability Service, Hunter New England Health Service, New South Wales 2298, Australia

[6]Grow-Up-Well Priority Research Centre, University of Newcastle, Newcastle, New South Wales 2308, Australia

Correspondence
Associate Prof. Cheryl Shoubridge, Discipline of Paediatrics, Adelaide Medical School, Faculty of Health Sciences, The University of Adelaide, Level 8 Adelaide Health and Medical School Building, Corner of George St and North Tce, S.A., South Australia 5005, Australia.
Email: cheryl.shoubridge@adelaide.edu.au

Communicating by María-Jesús Sobrido

Abstract

The *IQSEC2*- related disorders represent a spectrum of X-chromosome phenotypes with intellectual disability (ID) as the cardinal feature. Here, we review the increasing number of reported families and isolated cases have been reported with a variety of different pathogenic variants. The spectrum of clinical features is expanding with early-onset seizures as a frequent comorbidity in both affected male and female patients. There is a growing number of female patients with de novo loss-of-function variants in *IQSEC2* have a more severe phenotype than the heterozygous state would predict, particularly if *IQSEC2* is thought to escape X-inactivation. Interestingly, these findings highlight that the classical understanding of X-linked inheritance does not readily explain the emergence of these affected females, warranting further investigations into the underlying mechanisms.

KEYWORDS

affected females, escape X-inactivation, intellectual disability, IQSEC2, seizures

1 | *IQSEC2* GENE

IQSEC2 encodes the IQ motif and SEC7 domain containing protein 2 (*IQSEC2*) [NM_001111125] (MIM# 300522), which spans a 93.7 kb genomic region on chromosome X at Xp11.22. There are two major mRNA isoforms expressed from *IQSEC2*: the longer isoform contains 15 exons [GenBank: NM_001111125.2] and encodes a 1,488-residue protein (NP_001104595), whereas the shorter isoform contains 14 exons [GenBank: NM_015075.1] and encodes a 949-residue protein (NP_055890). These *IQSEC2* isoforms have 906 amino acids in common, which include a coiled-coiled domain (CC), a regulatory IQ-like motif, a catalytic Sec7 domain, and a pleckstrin homology (PH) domain, but vary at both the N- and C- termini (Figure 1). Some isoforms contain a C-terminal motif that can bind to type I PDZ domains. An alternative transcript for the longer *IQSEC2* isoform, resulting in a 1,478-residue protein, has also been proposed. A third isoform has been reported [GenBank:NM_001243197.1] that does not share any exons with the longest isoform, encodes a distinct, short 73-residue protein (NP_001230126.1), and as such is likely to have a distinct function and localization (Mignot et al., 2018). IQSEC2, also known as BRAG1 and IQ-ArfGEF, is a neuronally expressed ARFGEF for the small GTPase ARF6 (Murphy, Jensen, & Walikonis, 2006; Sakagami et al., 2008; Shoubridge et al., 2010) and is located at excitatory glutamatergic synapses in the forebrain. IQSEC2 interacts with postsynaptic density (PSD) proteins such as BAIAP2 (MIM# 605475) (Sanda et al., 2009) and the scaffolding protein known as DLG4 (MIM# 602887) via a C-terminal PDZ binding motif (Sakagami et al., 2008), forming a complex with *N*-methyl-D-aspartate (NMDA) receptors (recently reviewed in Um, 2017). The exact role IQSEC2 plays at excitatory synapses remains unclear, although the C-terminus of IQSEC2, which contains a PDZ-domain binding motif, appears to be involved in the activity-dependent removal of AMPARs (Brown et al., 2016). Certainly, both *Iqsec2* mRNA and protein have been identified in dendrites, suggesting that *Iqsec2* mRNA might be locally translated in an activity-dependent manner and contribute to synaptic plasticity (Sanda et al., 2009). However, our own studies have demonstrated that a reduction in Iqsec2 leads to

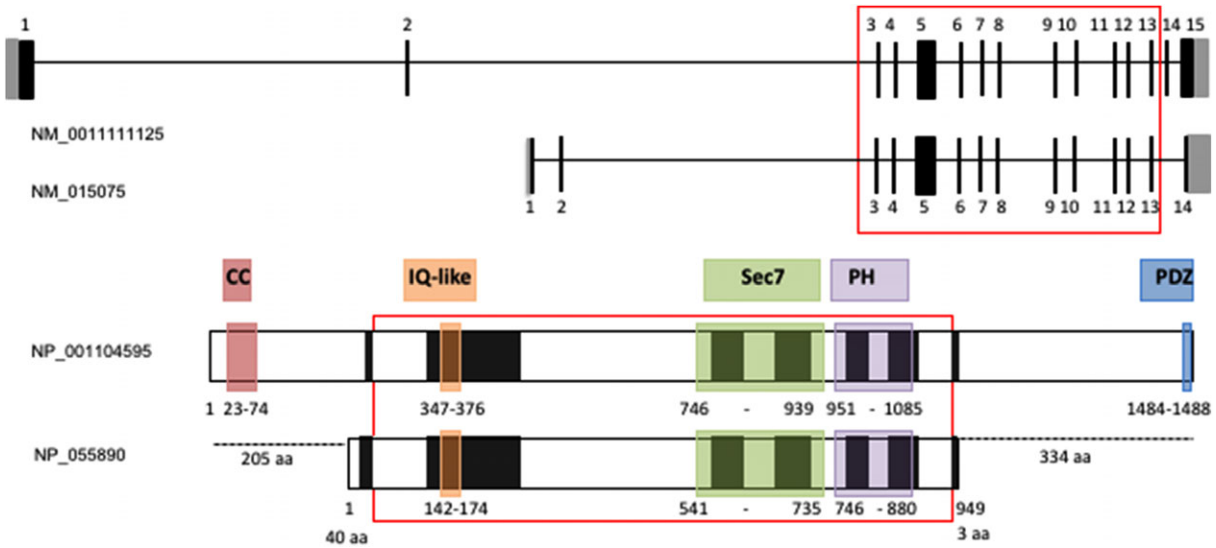

FIGURE 1 **Schematic of the two isoforms of *IQSEC2*.** The exon–intron structure of the longest isoform of *IQSEC2* gene [NM_0011111125.2] has 15 exons, with the ATG, open reading frame and stop codon positions in black and 5' and 3' untranslated regions in light grey. The shorter isoform contains 14 exons [GenBank: NM_015075.1]. The sequences of exon 3 to exon 13 are identical between the two isoforms (boxed with red line). Below is the predicted protein structure of the 1,488-residue protein (NP_001104595) with known functional domains highlighted; coiled–coiled (CC, red), IQ-like (orange), Sec 7 enzyme domain (Sec 7, green), PH domain (purple), and the PDZ-binding motif (blue). The corresponding amino acids are listed below each domain. Next is the 949-residue IQSEC2 isoform (NP_055890), containing three of the functional domains of the longer form. In comparison to the longer form of IQSEC2, the shorter isoform lacks 205 amino acids at the N-terminus, but contains 40 unique residues prior to the start of a region of identical sequence (boxed with red line) containing the IQ-like, Sec7 and PH domains, terminating three amino acids after the region of identical sequence, and lacks 334 amino acids from the C-terminal region compared to the longer protein form. This shorter protein therefore lacks both the N-terminal CC domain and the very C-terminal PDZ-binding motif

disturbed growth and morphology of developing neurons in cell culture (Hinze et al., 2017). There is currently no published research investigating the impact of loss or altered *Iqsec2* function on the development and resulting cognitive outcomes in any animal model.

2 | PATHOGENIC VARIANTS IN *IQSEC2*

The *IQSEC2* gene was first considered a candidate for X-linked neurologic disorders due to the identification of a de novo chromosomal translocation t (X; 20) (p11.2;q11.2) disrupting *IQSEC2* in a female patient with epileptic encephalopathy (EE) (Morleo & Franco, 2008). Disease-causing variants within *IQSEC2* were then identified in a large-scale X-exome re-sequencing study of non-syndromic intellectual disability (Tarpey et al., 2009). Functional validation of pathogenicity was required, as the reported variants were non-recurrent, non-synonymous SNPs. In four separate families with X-linked intellectual disability (XLID), we demonstrated that four different non-synonymous changes in the IQSEC2 IQ-like and Sec7 domains caused a reduction in ARFGEF activity (Shoubridge et al., 2010). Clinical features within these non-syndromic XLID families were moderate to severe intellectual disability in all affected males, with variable seizures, autistic traits and psychiatric problems (Shoubridge et al., 2010). Since this time, a total of 85 cases/families have been reported, including 37 novel variants in a recent report by Mignot and colleagues (Mignot et al., 2018). Overall, there are 70 different pathogenic variants arising from nine distinct variant types (Table 1), encompassing missense, nonsense, splice-site, deletions, indels, duplications, and several structural variations (SV). There are 10 cases of recurring pathogenic variants in nonrelated individuals, with each variant in only one additional case, with exception of two variants with four and five recurrent cases each.

The growing number of reported cases and families with *IQSEC2* pathogenic variants has seen an expansion in the spectrum of associated phenotypes. The inheritance patterns and the impact of different pathogenic variants on the clinical phenotypes in affected patients highlight several interesting trends. Of the 10 of 14 cases, half present with non-syndromic (NS) intellectual disability without seizures, with a preponderance of affected males (n = 49) compared to females (n = 22; Table 2). Females within these families are either asymptomatic carriers or mildly affected. This is in sharp contrast to the 65 cases due to de novo pathogenic variants where the reported frequency is similar in both males and females, and 81% of individuals have a phenotype that includes both intellectual disability and/or seizures (Table 2). In many cases, these de novo pathogenic variants have been identified due to unbiased high-throughput sequencing in cohorts of individuals with intellectual disability and/or epilepsy. Interestingly, several cases of affected females reported with de novo variants in *IQSEC2* were found in females included in studies based on a seizure phenotype, with limited clinical information on other comorbidities, including cognitive deficits. This highlights that pathogenic variants in *IQSEC2* may be an under-appreciated genetic cause of intellectual disability and, particularly, seizures in females. In addition to the comorbidity of seizures, there is a high prevalence of deficits to speech development and psychiatric features including autistic spectrum disorder (ASD) in both genders.

TABLE 1 Distribution of the type and frequency of pathogenic variants in *IQSEC2*

Variant Type *n* = 9	Number of variants *n* = 70	Number of families *n* = 85	Recurrent mutations (Variants/ families) *n* = 10 *n* = 25
Intragenic Variants			
Splice site	6	8	c.738-1G>C (2x)
			c.2582G>C (2x)
Nonsense	20	27	c.2272C>T (2x)
			c.2317C>T (2x)
			c.2776C>T (2x)
			c.3163C>T (4x)
			c.3433C>T (2x)
Missense	14	14	-
Deletions	17	22	c.804delC (5x)
			c.2052_2053 delCG (2x)
Insertions/deletions	3	3	-
Duplication	4	5	c.4039dupG (2x)
Structural Variants			
Balanced Translocation	1	1	-
SV(in-frame IQSEC2-TENM3 gene fusion)	1	1	-
Whole gene CNV	4	4	-

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence for the *IQSEC2* [GenBank: NM_001111125.2]
(#x) Number of families with each recurrent variation.

3 | GENOTYPE–PHENOTYPE RELATIONSHIPS OF PATHOGENIC VARIANTS IN *IQSEC2*

Despite the growing number of cases, the genotype–phenotype relationship for *IQSEC2* remains complex. The phenotype is influenced not only by the type of variant and how it is inherited, but also by the impact of a given variant on IQSEC2 function and the gender of the individual with the variant. Of the 64 distinct intragenic (coding) variants in 79 cases, there are 15 non-synonymous missense changes, one of which is also predicted to lead to a splice-site disruption (in two recurrent cases). Of these 15 missense variants, there are nine familial cases and seven cases arising de novo in patients (Table 3) and all disease-causing missense changes occur within specific functional domains of *IQSEC2* (with only one exception). Pathogenic variants are located within the IQ-like domain (Shoubridge et al., 2010; Zerem et al., 2016; Zhang et al., 2015), Sec7 domain (de Kovel et al., 2016; Gandomi et al., 2014; Helbig, *et al*., 2016; Helm et al., 2017; Kalscheuer et al., 2015; Mignot et al., 2018; Shoubridge et al., 2010), or PH domain (Helm et al., 2017; Mignot et al., 2018). The IQ-like and Sec7 functional domains are both involved in catalytic activity of guanine nucleotide exchange and activation of the substrate ARF6, while the PH domain may be involved in IQSEC2-accessory protein interactions, increasing the association of substrates (ARFGDP) with the catalytic Sec7 domain or recruiting IQSEC2 and associated signaling pathways to different subcellular compartments via interactions with phosphoinositides (Roy, Yohe, Randazzo, & Gruschus, 2016). Hence, the consequences of missense changes in these domains may cause a partial loss of IQSEC2 function either by impaired ARFGEF activity or mislocalization of IQSEC2 (Kalscheuer et al., 2015; Shoubridge et al., 2010). In general, affected male patients with missense variants present mild-severe non-syndromic XLID with variable penetrance of seizures and ASD traits (~24%) and less frequently with speech deficits. Most females with missense variants in the heterozygous state belong to families with affected males and are generally either asymptomatic carriers, or mildly affected with learning difficulties or borderline intellectual disability (ID; Table 3). There are at least three cases in which females with missense variants in *IQSEC2* were ascertained due to their seizure phenotype (de Kovel et al., 2016; Helbig, *et al*., 2016, Mignot et al., 2018) (Table 3). The only non-synonymous missense change found outside of any known functional domain was identified in an affected male with a phenotype of ASD, but no intellectual disability or seizures (Piton et al., 2011), representing the mildest reported phenotype within the IQSEC2 related disorders.

The remaining 49 coding pathogenic variants in 63 cases are due to nonsense variants, intragenic deletions, insertions, or duplications and splice-site variants, with the majority of cases being de novo in origin

TABLE 2 Inheritance and phenotype of intragenic coding variants in *IQSEC2*

		Phenotype		Males				Females (often limited information)			
Inheritance	Number of families/cases	ID	ID + Seizures		Seizures	Speech deficits	ASD traits		Seizures	Speech deficits	ASD traits
Familial	14	7	7	49	15	10	14	22	6	7	2
					30%	20%	28%		27%	22%	9%
De novo*	65	12	53	32	**29**	**28**	**11**	33	**24**	**22**	**12**
					90%	**87%**	**34%**		**72%**	**66%**	**36%**
	79	19	60	81	47	38	25	55	30	29	14
~136 Affected individuals					58%	46%	30%		54%	52%	25%

Does not include families with balanced translocations, structural variants (SV) or whole gene copy number variants (CNV) impacting IQSEC2.
*Includes 8 cases with "Unknown" or "not detected in mother, father unavailable" inheritance.

TABLE 3 Missense variants leading to intellectual disability and seizures occur in functional domains of *IQSEC2*

Variant cDNA	Ex	Variant protein	Domain	Inheritance	Family	Sex	Phenotype				Reference
							DD/ID	Seizures	Speech Deficits	Behavioral/Psychiatric/ physical	
c.1049C>T	4	p.Ala350Val	IQ-like	De novo	P1	M	Mod-Severe ID	EE	Regression in language	DevR, ASD and repetitive behavior, altered sensitivity to pain and sound	Zerem et al., 2016
c.1049C>A	4	p.Ala350Asp		Familial	3604	M	Severe ID	Partial, Febrile (6 mo)			Zhang et al., 2015
c.1075C>T	4	p.Arg359Cys		Familial	AU128	M (4)	Mild-mod ID	Seizures (2)		ASD traits (1)	Shoubridge et al., 2010
						F (6C*)	Mild ID (1), borderline ID (2)	Seizures (2)			
c.1688G>A	5	p.Arg563Gln	-	Familial	S0090	M				ASD traits	Piton et al., 2011(S)
c.2273G>A	5	p.Arg758Gln	Sec7	Familial	US166	M (8)	Mild ID	None	No speech until 6 yrs (1)	No ASD traits	Shoubridge et al., 2010
						F (7C*)		None	None	No phenotype reported	
c.2278G>A[a]	5	p.Gly760Ser		De novo	P21	F	Mod ID	None	None	Hyperactivity	Mignot et al., 2018
c.2312G>A[a]	6	p.Gly771Asp		De novo	EP1961	F	Severe ID	Focal seizures (14 mo)	Sentence 2–3 words (24 yrs)	Mild autistic behavior	de Kovel et al., 2016 Mignot et al., 2018
					P22						
c.2354C>T[a]	6	p.Pro785Leu		De novo	P25	M	Severe ID	None	Non-verbal		Mignot et al., 2018
c.2366C>T	6	p.Ala789Val		Familial	MRX78	M (6)	Severe ID (5), Mod ID (1)	Seizures (2) Intractable (1)		Ag (3), ASD traits (3)	Kalscheuer *et al*., 2016
						F (6C*)	Mild ID (4), LD (2)				
c.2402A>C	6	p.Gln801Pro		Familial	MRX18	M (8)	Mod-severe ID	Seizures (2)		ASD traits (4)	Shoubridge et al., 2010
						F (6C*)	Borderline ID (3)			Psychiatric features (1)	
c.2507C>T	7	p.Ala836Val		Familial	P1	M	Global DD	Seizures - intractable	Non-verbal (3 yrs)	Poor social interaction, spasticity of limbs, MRI transient events	Helm et al., 2017

(*Continues*)

TABLE 3 (Continued)

Variant cDNA	Ex	Variant protein	Domain	Inheritance	Family	Sex	Phenotype				Reference
							DD/ID	Seizures	Speech Deficits	Behavioral/Psychiatric/physical	
c.2582G>C	7	p.Ser861Thr (splice)		De novo	P2	M	DD	EE - intractable (2 yrs)		Stereotypic hand movements, hypoplastic corpus callosum by MRI	Gandomi et al., 2014
					46	F		Seizures		Limited phenotype reported	Helbig et al., 2016
c.2587C>T	8	p. Arg863Trp		Familial	MRX1	M (12)	Mod ID	Seizures (1)		No ASD traits, Psychiatric features (1)	Shoubridge et al., 2010
						F (6C*)	Borderline ID (3)	None		Psychiatric features (1)	
c.2995C>T	10	p.Leu999Phe	PH	De novo	P5	M	DD/Severe ID	Early-onset epilepsy (2 yrs)	Virtually non-verbal (9 yrs)	(9 yrs) Non-ambulatory, self-harming	Helm et al., 2017
c.3206G>C[a]	12	p. Arg1069Pro		Familial	P33	M	Severe ID	EE (1 yr)	Non-verbal (6.9 yrs)	Autistic behavior	Mignot et al., 2018

Abbreviations used: Ag, aggression; ASD, autistic spectrum disorder; C*, carrier female within X-linked family; DevR, developmental regression; DD, developmental delay; ID, intellectual disability; LD, learning difficulties; Mod, moderate.

Numbers (alone) in brackets indicate the number of affected individuals, mo = months, yrs = years.

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence for the *IQSEC2* [GenBank: NM_ 001111125.2].

[a]Cases listed as "likely pathogenic" in Mignot et al., 2018.

(Table 4). There are two de novo in-frame deletions, each removing one amino acid within the coiled–coiled domain. The female case was reported with a Rett-like syndrome phenotype, including typical loss of language (Sajan et al., 2017). In the male case, severe ID, and partial seizures were reported (Zhang et al., 2015). There is an in-frame deletion/insertion leading to a predicted loss of 210 amino acids and insertion of a glycine between the IQ-like and Sec7 domains, in a male patient with severe ID, infantile spasms, and nonverbal and autistic behavior (Mignot et al., 2018). The remaining cases lead to predicted premature termination codons either at the site of the variant or due to frameshifts of the codon sequence arising from nonsense variants and intragenic deletions, insertions, or duplications. However, the genotype–phenotype relationship is complicated by the impact of these variants on protein function and the gender in which the variant is expressed. Specifically, several variants lead to the premature termination codon being located within 50 nucleotides of the subsequent exon junction. In these cases, we cannot rule out that the resulting mutant transcripts may escape nonsense-mediated RNA decay. If this occurs, there is the potential for a partial protein that is C-terminally truncated to be produced from these mutant alleles. Interestingly, the six variants in this category would truncate the IQSEC2 protein after a residue that is critical for Sec7 catalytic activity (p.E849) (Beraud-Dufour et al., 1998). In this setting, an affected male in the hemizygous state might escape complete loss of IQSEC2 function resulting in an attenuated phenotype. The impact on females is less easy to predict. For example, there are two variants in males presenting with severe ID but no seizures (Rauch et al., 2012; Tran Mau-Them et al., 2014; Tzschach et al., 2015). Similarly, a splice-site variant in the PH domain presents with moderate to severe ID in males and mild ID in carrier females, with no history of seizures (Madrigal et al., 2016). The variant activates an exonic splice acceptor site, producing a deletion spanning 70 nucleotides in exon 12. This results in the loss of 26 amino acids from the PH domain and a premature termination codon after another 36 amino acids. PCR analysis from exon 9 to 12 in this family indicated that two separate products were made: a normal product and a truncated version due to the variant. Hence, only a proportion of *IQSEC2* transcripts were mis-spliced, and the ratio of the normal versus mutant product was suggested to underpin the severity of phenotype in this family. Two other variants that could be considered in this category were reported in patients with severe ID, seizures, and severe language deficits, in affected females (Ewans et al., 2017) and affected males (Redin et al., 2014). Interestingly, one of these cases was inherited in four affected sisters, all with moderate-severe ID, epilepsy, and deficits to speech with variable penetrance of some behavioral disturbances (Ewans et al., 2017). This variant was not detectable in genomic DNA from blood in either parent, consistent with inheritance as a consequence of gonadal mosaicism (Ewans et al., 2017).

The majority of pathogenic variants are predicted to lead to premature termination that is subject to the RNA surveillance process of nonsense-mediated decay, leading to a complete loss of mutant IQSEC2 protein (Table 4). These variants occur across all regions of IQSEC2 and are not constrained to particular functional domains. Male patients with loss of function variants invariably present with severe

TABLE 4 Intragenic nonsense, duplication/truncation, in-frame deletions, and splicing variants in *IQSEC2* leading to intellectual disability and seizure phenotypes

							Phenotype				
Variant cDNA	Ex	Variant Protein AA	Domain	Inheritance	Case	Sex	DD/ID	Seizures	Speech deficits	Behavioral/Psychiatric/ physical features	Reference
c.55_151delinsAT	1	p.Ala19Ilefs*32	–	De novo	P1	F	Mild ID	None	Pronunciation, syntax issues at 6.5 yrs	Tantrums, anxiety	Mignot et al., 2018
c.83_85del	1	p.Asp28del	CC	De novo	108286	F	Rett-like	None	Loss of language	Regression-stabilization, gait abnormalities	Sajan et al., 2017
c.88_90delATC	1	p.Ile30del		De novo	3481	M	Severe ID	Partial seizures (3 yrs)	–	–	Zhang et al., 2015
c.97C>T	1	p.Gln33*		De novo	P2	M	Severe ID	GTCS (7 yrs)	Nonverbal at 11 yrs	Autistic behavior, unexplained laughter, no communication, stereotypic activates	Mignot et al., 2018
c.184C>T	1	p.Arg62*		De novo	P3	M	Severe ID	Seizures (3 yrs) atonic, myoclonic, tonic, absences	Non-verbal at 12 yrs	Self-injurious behavior, strabismus	
c.273_282del	1	p.Asn91Lysfs*112	–	De novo	P7	F	Rett-like	None reported	Partial or loss of spoken language	Regression-stabilization, gait abnormalities, stereotypic hand movements, inappropriate laughing/screaming spells.	Olson et al., 2015
c.577_610del	1	p.Arg197Alafs*34	–	De novo	P4	M	Profound ID	IS (8 mo), tonic, GTCS, atypical absences	Non-verbal at 9.5 yrs	Autistic behavior, strabismus, MRI cerebral atrophy	Mignot et al., 2018
c.738-1G>A	Int 2	Splicing	–	Familial	P5, P6	M (2)	Severe ID	EE (5 mo)	2-3 word sentences (12.5 and 9.5 yrs)	Autistic behavior	
				De novo	P7	M	Severe ID	Seizures (11 mo)	1 word at 3.5 yrs	-	
c.804delC	3	p.Try269Thrfs*3	–	De novo	48	F		Seizures		Limited phenotype reported	Helbig et al., 2016
					P2	M	Severe ID	Seizures	Non-verbal	ASD traits	Helm et al., 2017
					P8	M	Severe ID	Seizures (15 yrs)	Virtually non-verbal		Mignot et al., 2018
					P9	M	Severe ID	LGS (27 mo)	Non-verbal at 10 yrs	Dyskinesia	
					P10	M	Severe ID	LGS (5 yrs)	Non-verbal at 22 yrs	Spastic quadriparesis	Mignot et al., 2018, Hamdan et al., 2017

(Continues)

TABLE 4 (Continued)

Variant cDNA	Ex	Variant Protein AA	Domain	Inheritance	Case	Sex	Phenotype				Reference
							DD/ID	Seizures	Speech deficits	Behavioral/Psychiatric/ physical features	
c.854del	3	p.Pro285Leufs*21	–	De novo	P11	F	Severe ID	Seizures (12 mo) tonic-clonic	Says words at 16 yrs	Limb rigidity, walking instability	Mignot et al., 2018
c.895C>T	3	p.Gln299*	–	De novo	P12	M	Profound ID	IS (10 mo) GTCS, myoclonic jerks, absences	Non-verbal at 20 yrs	MRI mild cerebral atrophy, strabismus	Mignot et al., 2018, Hamdan et al., 2017
c.928G>T	3	p.Glu310*	–	De novo	P16	F	Mild-mod ID	FE	2 yrs.11 mo says a few words	No ASD or other features.	Zerem et al., 2016
c.1000_1034del	Skip Ex4	Splicing	–	De novo	P3	M	Global DD Severe ID	Seizures (8 yrs)	Virtually non-verbal	5 yrs. microcephaly – 6 yrs. global cerebral atrophy and white matter thinning,19 yrs. wheelchair bound	Helm et al., 2017
DupX:53283513-53325282	E2–E4	–Dup/truncation		De novo	P1	M	Severe ID	EE- Generalised Myoclonic seizures (4 yrs)	Non-verbal at 4 yrs	4 yrs. cannot walk alone, stereotypic hand movements, self-injury, strabismus	Tran Mau-Them et al., 2014
DupX:53276030-53298472	Int 2–E8	–Dup/truncation		De novo	P3	M	severe ID	Partial epilepsy (3 yrs)	Language regression	Midline stereotypic hand movements ~ 3 yrs, strabismus, self-injury, MRI cerebral atrophy	
c.1405_1406del	5	p.Lys469Valfs*4	–	De novo	P13	M	Profound ID	Seizures (3 mo) atonic, spasms	Non-verbal at 11 yrs	Global hypotonia, spasticity of 4 limbs	Mignot et al., 2018
c.1510C>T	5	p.Gln504*	–	Unknown	P14	M	Profound ID	Seizures (2 yrs)	Non-verbal at 5.8 yrs	Global hypertonia,	
c.1567_2199 delinsGGC	5	p.Thr523_Thr 733delinsGly	–	De novo	P15	M	Severe ID	IS (30 mo), GTCS, tonic, spasms	Non-verbal at 5.3 yrs	Autistic behavior, hypertonia,	
c.1618C>T	5	p.Gln540*	–	De novo	P14	M	DD Severe ID	EE	Non-verbal at 3 yrs	3 yrs. Not sitting, Hypertonia, ASD traits, microcephaly, Strabismus, Hypomyelination and thin corpus callosum	Zerem et al., 2016
c.1744_1763del	5	p.Arg582Cysfs*9	–	De novo	P16	F	Mild DD	Focal epielpsy (17 mo)	50-60 words at 3 yrs	Autistic behavior, hypertonia,	Mignot et al., 2018

(Continues)

TABLE 4 (Continued)

Variant cDNA	Ex	Variant Protein AA	Domain	Inheritance	Case	Sex	Phenotype				Reference
							DD/ID	Seizures	Speech deficits	Behavioral/Psychiatric/ physical features	
c.1983_1999del	5	p.Leu662Glnfs*25	–	Unknown	P17	F	Global DD	Focal epilepsy (11 mo)	Babbling at 16 mo	Hypertonia	
c.2052_2053 delCG	5	p.Cys684*	–	De novo	P1	M	DD	EE - intractable	Non-verbal at 4 yrs	Non-ambulatory, strabismus, hand flapping and limited interest in relationships, MRI small brain relative to cranium	Gandomi et al., 2014
					47	F		Seizures		Limited phenotype reported	Helbig et al., 2016
c.2078delG	5	p.Gly693Val*29	–	Unknown	P18	F	Mod global DD	None	Non-verbal at 2.8 yrs	Self-injurious behavior, hypotonia	Mignot et al., 2018
c.2203C>T	5	p.Gln735*	–	De novo	T17563	F	Mild ID	SGE (5 yrs)—regression with non-convulsive SE. Absence to tonic-clonic and myoclonic seizures, drop attacks. Offset at 38 yrs			Epi4K Consortium, 2016
c.2272C>T	5	p.Arg758*	–	De novo	P19	F	Severe ID	Multifocal epilepsy (23 mo)	3 words at 11.3 yrs	Self-injurious behaviors	Mignot et al., 2018
					P20	F	Mod ID	Seizures (9 yrs 4 mo) GTCS, focal, atypical absences	Speaks sentences, reasoning difficulties		
c.2317C>T	6	p.Gln773*	Sec7	De novo	P6	F	Global DD	Seizures (18 mo)		Hypotonic, strabismus, dysmorphic face	Helm et al., 2017
				Unknown	P23	F	Mod ID	Seizures (14 yrs), GTCS, absences	Few words at 43 yrs	ASD (13 yrs) aggressive	Mignot et al., 2018
c.2317_2332del	6	p.Gln773Glyfs*25		Unknown	P24	F		Seizures (6 yrs)	Sentences at 11.3 yrs		
c.2563C>T	7	p.Arg855*		De novo		M	Severe ID	None	Speech not achieved by 3 yrs	Strabismus, ASD traits, avoiding eye contact, repetitive hand movements	Rauch *et al*., 2014 Tran Mau-Them et al., 2014

(Continues)

TABLE 4 (Continued)

Variant cDNA	Ex	Variant Protein AA	Domain	Inheritance	Case	Sex	Phenotype				Reference
							DD/ID	Seizures	Speech deficits	Behavioral/Psychiatric/ physical features	
c.2662dup	8	p.Ile888Asnfs*16		De novo	P7	M	Severe XLID			No other significant clinical problems	Tzschach et al., 2015
c.2679_2680insA	8	p.Asp894fs*10		Familial	K2	F (4)	DD, Mod-severe ID	Epilepsy	Non verbal (2) language delay (1)	Ag behavior (young) ASD traits (2)	Ewans et al., 2017
c.2776C>T	9	p.Arg926*		De novo	P3	F	Severe ID Rett-like	EE	Speech delay, language regression at 2 yrs. Now non-verbal	Autistic behavior (balance & hand sterotypies), pain sensitivity & aggressive	Allou et al., 2017
					P26	F	Profound ID	LGS (23 mo)	Non-verbal at 11.3 yrs	Autistic behavior, truncal hypotonia, strabismus	Mignot et al., 2018
c.2799C>G	9	p.Try933*		De novo	M2189	F	Global DD Mod ID		Speech delay, non-verbal at 14 yrs	ASD, sleep disturbances, behavioral aspects, oral motor dyspraxia, strabismus	Berger et al., 2017
c.2846_2852 delCCCAGGT	9	p.Ser949Cysfs*7	PH	De novo	5292	M	Severe ID	Partial seizures, Infantile spasms			Zhang et al., 2015
c.2854C>T	9	p.Gln952*		De novo	P27	F	Severe ID	EE (12 yrs), absences, GTCS	Nonverbal at 16 yrs	Autistic behavior, dystonia, tremor, ataxia	Mignot et al., 2018
c.2962C>T	10	p.Gln988*		De novo	P28	M	Severe ID	IS (20 mo), spasms, myoclonic jerks, tonic, atonic	Nonverbal at 6.9 yrs	ASD, self-injurious behavior, hypotonia, strabismus, MRI cerebral atrophy, hypomyelination	
c.3079delC	11	p.Leu1027Serfs*75		De novo	P29	F	Mod - severe ID	None	10 words at 8 yrs		
c.3097C>T	11	p.Gln1033*		De novo	APN-68	M	DD severe ID	EE	No acquired language	Strabismus, stereotypic features, and behavioral disorder	Redin et al., 2014
g.88032_ 88033del	Int 11	Splicing		Familial		M (3)	Mod - severe ID	None	Speech delay	No ASD traits, obesity, and short stature	Madrigal et al., 2016
						F (3C*)	Mild ID (2) LD (1)	None	Language delay (2)	No ASD traits	

(Continues)

TABLE 4 (Continued)

Variant cDNA	Ex	Variant Protein AA	Domain	Inheritance	Case	Sex	Phenotype				Reference
							DD/ID	Seizures	Speech deficits	Behavioral/Psychiatric/ physical features	
c.3163C>T	12	p.Arg1055*		De novo	Pat 19	F	Severe ID	Epilepsy		Borderline macrocephaly, skewed X-inactivation (97:3)	Tzschach et al., 2015
				Familial	P30	M	Severe-profound ID	EE (2 yrs) drop attacks, tonic, myoclonic jerks	Nonverbal at 7 yrs	Global hypotonia, mild thinning of corpus callosum, mild cerebral atrophy	Hamdan et al., 2017
											Mignot et al., 2018
				Unknown	P31	F	Mod - severe ID	Seizures (5 yrs 8 mo) GTCS, focal dyscognitive	3 word sentences, and 20 words at 8 yrs	Autistic behavior, Global hypotonia, aggression, hyperactivity	Mignot et al., 2018
				De novo	P32	M	Profound ID	EE (13 yrs) spasms, atonic, atypical absences, myoclonic, GTCS	Nonverbal at 13 yrs	Autistic and self-injurious behavior, hypotonia, spasticity, cerebral and cerebellar atrophy, hypomyelination	
c.3277+2T>G	Int 12	Splicing	–	De novo	P34	M	Profound ID	LGS (3 yrs) atonic falls, tonic, atypical absences, late-onset spasms	Nonverbal at 15 yrs	Autistic behavior, stereotypic and self-injurious behavior, dystonia. mild cerebral atrophy, thin corpus callosum	
c.3277+5G>A	Int 12	Splicing	–	Familial	P35	M	Severe ID	IS (13.5 mo)	Nonverbal at 6 yrs	Global hypotonia	
c.3278C>A	13	p.Ser1093*	–	De novo	P36	F	Severe ID	None	Few words, rare sentences at 13 yrs		Mignot et al., 2018
c.3322C>T	13	p.Gln1108*	–	De novo	KO	F		EE			Epi4K Consortium, 2013
c.3387C>A	13	p.Tyr1129*	–	De novo	D12136, P37	M	Severe-profound ID	EE (3 yrs) atonic falls, absences	Nonverbal at 3.5 yrs	Autistic behavior, self-injurious behavior, hypotonia, strabismus. MRI cerebral atrophy, thin corpus callosum (5 yrs)	de Kovel et al., 2016, Mignot et al., 2018

(Continues)

TABLE 4 (Continued)

Variant cDNA	Ex	Variant Protein AA	Domain	Inheritance	Case	Sex	Phenotype				Reference
							DD/ID	Seizures	Speech deficits	Behavioral/Psychiatric/ physical features	
c.3433C>T	13	p.Arg1145*	–	De novo	P38	M	Profound ID	Seizures (10 mo) upward eye deviations ± absence and myoclonic jerks	Nonverbal at 9.8 yrs	Global hypotonia	Mignot et al., 2018
				Not in mother, Father unavailable	P39	F	Severe ID	Focal epilepsy (11 mo) focal, tonic, tonic-clonic	Nonverbal at 11 yrs	Autistic behavior	Mignot et al., 2018
c.3457del	14	p.Arg1153 Glyfs*244	–	De novo	P40	F	Severe ID	IS (7 mo) spasms, focal, absence, tonic, myoclonic jerks	Nonverbal at 20 yrs	Autistic behavior, truncal hypotonia. MRI mild atrophy, and cerebral white matter hyperintensities	Mignot et al., 2018
c.3669_3733del	15	p.Thr1225SerFs*4 (64bp del)		De novo	P4	M	Severe ID DevR	Seizure onset (8 mo) Intractable seizures (20 yrs)	Nonverbal	Seizure 8 mo, no dev beyond this (20 yrs now), fed by tube, cerebral atrophy MRI, non-ambulatory	Helm et al., 2017
c.4039dupG	15	p.Ala1347 Glyfs*40	–	De novo	1098M	F		EE (onset 19 mo)		ASD, Macrocephaly	Parrini *et al.*, 2016
					P41	F	Mild-mod ID	Seizures (3 yrs) absence and falls	Speaks sentences, writes first name, counts to 15 at 11 yrs	Mild autistic behavior	Mignot et al., 2018
c.4401del	15	p.Gly1468 Alafs*27	–	Not in mother, Father unavailable	P42	F	Mod-severe ID	None	Short sentences at 11 yrs	Attention deficit/hyperactivity	

Abbreviations used: Ag, aggression; ASD, autistic spectrum disorder; C*, carrier female within X-linked family; DevR, developmental regression; DD, developmental delay; EE, epileptic encephalopathy; GTCS, generalized tonic clonic seizures; ID, intellectual disability; IS, infantile spasms; SGE, symptomatic generalised epilepsy.

Del, deletion; dup, duplication; mo, months; yrs, years. Numbers (alone) in brackets indicate number of affected individuals.

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence for *IQSEC2* [GenBank: NM_001111125.2].

ID, seizures, and are essentially nonverbal (Gandomi et al., 2014; Helbig et al., 2016; Helm et al., 2017; Mignot et al., 2018; Tran Mau-Them et al., 2014; Zerem et al., 2016). Female patients with loss of function variants in the heterozygous state present with a spectrum of severity in intellectual disability spanning from global developmental delay (in younger patients) through to mild ID and in a few cases, severe ID (Allou et al., 2017; Berger et al., 2017; Epi et al., 2013; Epi4K Consortium, 2016; Helbig et al., 2016; Helm et al., 2017; Mignot et al., 2018; Olson et al., 2015; Parrini et al., 2017; Tzschach et al., 2015; Zerem et al., 2016). Seizures were reported in 20 of 29 cases of these affected females, with 19 females reported to have speech delay or loss of spoken language, and eight with ASD. Unsurprisingly, these variants all occur in a de novo manner, which likely reflects reduced reproductive fitness in affected females.

4 | STRUCTURAL VARIANTS OF *IQSEC2*

IQSEC2 was first considered a candidate gene for X-linked neurologic disorders due to the identification of a de novo chromosomal translocation t(X;20)(p11.2;q11.2) disrupting *IQSEC2* in a female patient with epileptic encephalopathy (EE) (Morleo & Franco, 2008). An unusual structural variant identified using whole-genome sequencing resulted in a partial duplication of *TENM3* (MIM# 610083) exons 22 to 27 on chromosome 4 that was inserted into *IQSEC2* (Gilissen et al., 2014). This resulted in the formation of a stable in-frame *IQSEC2-TENM3* gene fusion that disrupts IQSEC2, contributing to the patient's phenotype of severe ID (Table 5). Copy number variants impacting Xp11.22 include submicroscopic duplications at Xp11.22 containing the known ID genes *IQSEC2*, *KDM5C* (MIM# 314690), and *TSPYL2* (MIM# 300564) in four male patients with ID (Moey et al., 2016) and microdeletions of *KDM5C* and *IQSEC2* in a female patient (Fieremans et al., 2015) (Table 5).

The identification of variants in known intellectual disability genes that are novel and potentially pathogenic are increasingly being reported in large-scale screening studies or reports from diagnostic and clinical laboratories. In some cases, these variants are published with very limited clinical phenotype data. For *IQSEC2*, there are at least 10 additional variants from a range of such studies (listed on Supporting Information Table 1) for which phenotypic data, often including gender of the patient, is not stated. For large-scale genomic studies, this highlights the need for a minimal amount of clinical data to be available for all patients within a cohort.

Considering the current pathogenic variants collectively, it is clear that a complete loss of *IQSEC2* in the hemizygous male state may not be lethal but leads to the most severe clinical presentations of severe ID, invariable seizures in patients that are essentially nonverbal (Figure 2). In comparison, affected females particularly with missense variants inherited across families are generally less severely affected than males. However, the high prevalence of intellectual disability and early onset-seizures in females with de novo loss of function varaints is still more severe than would be expected from variants found in the heterozygous state in a gene on the X-chromosome.

5 | TOLERATED VARIATION IN *IQSEC2*

The wealth of publicly available data cataloging population-level variation in individual genes has been a welcome and necessary response to the exponential capacity to screen the human genome at increasing levels of resolution. Resources such as the Exome Aggregation Consortium (ExAC) and the Genome Aggregation database (gnomAD) consist of 123,136 exome sequences and 15,496 whole-genome sequences from unrelated individuals sequenced as part of various disease-specific and population genetic studies (Hu et al., 2016). Variation within individual genes examined at a population level provides pivotal information that can help identify which variants detected in patients are likely to be tolerated versus those that are likely to contribute to disease. The levels of variation and types of predicted impact on the resulting gene/protein function that are observed in the population, compared to what would be expected based on the size and complexity of each gene, highlights that some genes and gene regions are less tolerant of variation. In the case of *IQSEC2*, the number of observed synonymous variants are as expected (87.6 expected versus 94 observed; Table 6). However, there is a significant reduction in the observed number of LoF (expected 15.5, observed 1) and missense variants (expected 221.9, versus 86 observed with an allele frequency of <0.0001) than would otherwise be expected based on size and complexity of the gene (Table 6, top panel). In an attempt to identify regions of the gene/protein that have different thresholds of tolerated variation, the type and frequency of variation across each of the genotype states (hemizygote males, and homozygote or heterozygote females) is summarized in Table 6, bottom panel. The more severe stop-gained and LoF variants each have a single case from over 60,000 individuals sequenced, with no cases reported in hemizygous males or homozygous females. Similarly, there is only one case of a splice donor and in-frame insertion reported, each in a single hemizygous male. Even missense variation is limited in this gene, and there are no missense variants reported in the homozygous state in females. There are eight missense variants reported in more than two (but no more than 15) hemizygous males, and 36 variants each with only a single hemizygous case. Each of the missense variants are depicted in Figure 2 underneath a protein schematic at the location of the amino acid impacted, and are stratified by the allele frequency and number of hemizygous males. Analysis of the larger data set (Supporting Information Table 2) capturing both the combined exomes and genomes sequenced as part of ExAC and gnomAD highlights a frequent missense variant in over 440 alleles, including 89 hemizygote males and three homozygote females at p.Ala1470Thr. Apart from this exception, a restricted level of tolerated variation (particularly in hemizygote males) is also observed in this broader data set (Supporting Information Table 2).

6 | MOLECULAR PATHOLOGY OF PATHOGENIC VARIANTS IN *IQSEC2*

To understand how variants in *IQSEC2* contribute to disease, we need to consider not only the role of IQSEC2 in activating the ARFs (ADP

TABLE 5 Genotype-phenotype of balanced translocation and whole-gene CNVs involving *IQSEC2*

Patient	Chromosome X variant coordinates and size (GRCh37/Hg19)	XLID genes duplicated/deleted	XLID genes disrupted	Sex	Phenotype			Reference
					DD/ID/seizures	Speech deficits	Behavioral/Psychiatric features/physical features	
AD	Balanced translocation 46,X,t(X;20) (p11.2;q11.2)		*IQSEC2* (long isoform)	F	DevR, Epilepsy (myoclonic, onset 15 mo)	Speech regression with onset of seizure	Hypotonic, EEG consistent with hypsarrthymia, brain MRI show non-specific volume loss, and periventricular white matter changes	Morleo & Franco, 2008
Patient 31 (*55)	Dup on chr4: g.183693432_183756173dup GAIN 62kb Insertion point on chrX g.53318362_53318363	*Last 6 exons of TENM3 are duplicated*	*IQSEC2* (long isoform)	F	Severe ID Epilepsy (tonic clonic ~ onset 18 yrs.)	Non-verbal	Microcephaly, progressive spasticity, small hands and feet, poor vision, hypotonic	Gilissen et al., 2014 *De Ligt, *et al*., 2012
P1	g.52954520_53315542dup GAIN 361kb	*TSPYL2*, *KDM5C*, *IQSEC2* (short isoform)	*IQSEC2* (long isoform)	M	DD	Severe speech delay	Poor socialization	Moey et al., 2016
Family 2	g.52911287_53315010dup GAIN 403kb			M (III-1)	DD LD	No speech delay	ASD-F, physical aggression when frustrated, obsessive tendencies, dyspraxia	
				(III-4)	LD		ADHD as a child, obsessive tendencies, dyspraxia	
				(III-6)	Moderate LD	Problems with expressive and receptive speech	ASD-F, ADHD as a child, obsessive behavior	
P3	g.52789239_53368927dup GAIN 579kb	*TSPYL2*, *KDM5C*, *IQSEC2*		M	DD Seizures	Severe expressive speech delay	Behavioral problems requiring a special education class	
P1	g.52920728_53321125del LOSS 0.4Mb		*IQSEC2* (long isoform)	F	Severe ID	Non-verbal	ASD-F, poor eye contact and socialization	Fieremans et al., 2015

Abbreviations used: ADHD, attention deficit hyperactivity disorder; ASD-F, autistic features; DD, developmental delay; DevR, developmental regression; ID, intellectual disability; LD, learning difficulties.

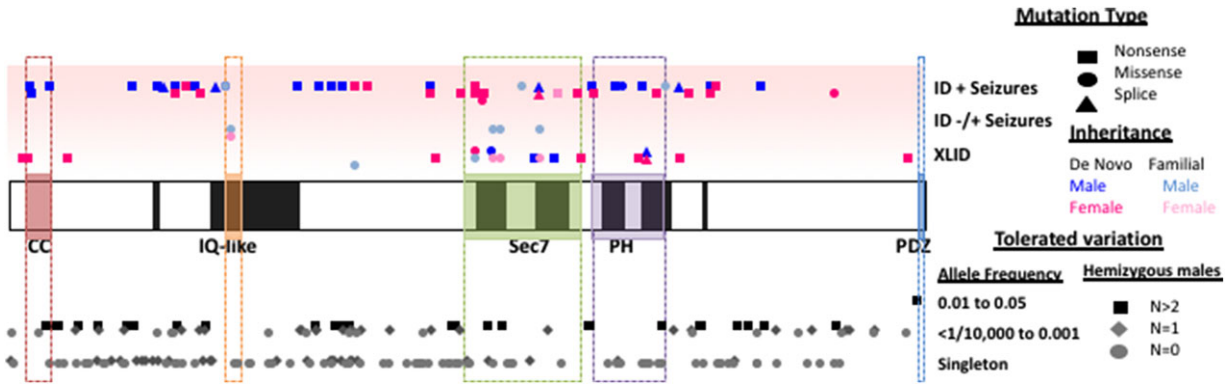

FIGURE 2 **Summary of the type, location, and phenotypes due to pathogenic variants in *IQSEC2*.** Predicted protein structure of the 1,488-residue protein with coiled–coiled (CC, red), IQ-like motif (orange), catalytic ArfGEF/Sec7 domain (green), PH domain (purple), and PDZ-binding motif (blue). The variants are depicted above the region of impact on the predicted protein structure. Nonsense (square), missense (circle), and splice (triangle) variants in affected males (blue) and females (pink) with de novo cases in dark colors and familial cases in pale colors. Variants are ranked against severity of clinical phenotype

TABLE 6 Tolerated variation in *IQSEC2* from the ExAC

Constraint from ExAC	Expected no. variants	Observed no. variants (AF <0.001)	Constraint Metric
Synonymous	87.6	94	z = –0.42
Missense	221.9	**86**	z = 4.46
LoF	15.5	**1**	pLi = 0.98

Variation listed in ExAC	Total No.	Allele count	Allele Freq	No. Homozygotes	No. Hemizygotes
LoF	1	1	0.00002316	0	0
In frame deletion	1	42	0.003241	0	0
In frame insertion	1	2	0.0001544	0	1
Stop gained	1	1	0.00001258	0	0
Splice donor	1	1	0.00001366	0	1
Missense	8	2–49	0.01125 to 0.00002976	0	2–15
	36	1–4	0.0003162 to 0.00001148	0	1
	61	1–4	0.0003067 to 0.00001143	0	0

a. Mean coverage of 23.45 of canonical transcript: ENST00000396435.
b. UCSC browser X:53260258-53350522.

ribosylation factors) family GTPases, but also how diminished activation of ARFs may in turn contribute to dysfunction. ARFs are members of the Ras superfamily of small G proteins and despite their name, are not involved in ADP-ribosylation or regulation of heterotrimeric G proteins. ARFs have a primary role in the regulation of vesicular transport, organelle structure and participate in membrane traffic and organization of the cytoskeleton (reviewed in Casanova, 2007; Gillingham & Munro, 2007; Kahn et al., 2006). Mammals have six ubiquitously expressed genes/paralogs, divided into three classes based on sequence similarity (Class I: Arf1-3, Class II: Arf4-5, Class III: Arf6). Class I and II ARFs are localized to the Golgi and endosomal compartments, whereas ARF6 (MIM# 600464) is associated with the plasma membrane and a subset of endosomes at the cell periphery (D'Souza-Schorey & Chavrier, 2006). The ARF proteins switch between GTP-bound active and GDP-bound inactive conformations. GTP-activating proteins (GAPs) mediate the hydrolysis of bound GTP while the exchange of GDP for GTP nucleotide is mediated by guanine nucleotide exchange factors (GEFs).

There are 15 ARFGEFs in the human genome which all share an approximately 200 amino acid Sec7 domain responsible for catalyzing nucleotide exchange. These ARFGEFs include the GBF/BIG, Cytohesins, EFA6, Fbox, and the IQSEC proteins, generally with multiple family members (Casanova, 2007; Gillingham & Munro, 2007; Kahn et al., 2006). The catalytic mechanism of this domain revolves around an invariant glutamate residue located at the tip of the hydrophilic loop between the sixth and seventh of 10 transverse α-helices, referred to as a glutamic finger. It is this glutamate that is inserted into the nucleotide-binding fold of the ARF protein to compete electrostatically with the β-phosphate of the bound nucleotide (Beraud-Dufour et al., 1998).

ARF proteins operate by the classical GDP/GTP exchange common to all G proteins (Vetter & Wittinghofer, 2001), which they couple to a cytosol-membrane translocation (Pasqualato, Renault, & Cherfils, 2002). This mechanism is based on an extended switch region encompassing the switch 1 and 2 regions (residues 38–52 and 69–84 in ARF1) and the intervening interswitch region comprising strands β2 to β3

(Renault, Christova, Guibert, Pasqualato, & Cherfils, 2002). When the ARF protein is located in the cytosol, the interswitch is locked in a retracted conformation that blocks nucleotide exchange and the binding of GTP (Amor, Harrison, Kahn, & Ringe, 1994; Menetrey, Macia, Pasqualato, Franco, & Cherfils, 2000). Binding of ARF reversibly to membranes leads to an 'interswitch toggle', initiating a conformation that can bind GTP (Goldberg, 1998). It is this step that is stimulated by the GEFs that contain a Sec7 domain with the invariant glutamate. The process of nucleotide exchange on ARF proteins occurs in an ordered series of steps. First, the switch 1 and 2 domains of the ARF protein are prised open by Sec7 domain, and this induces a rotation of the ARF protein core such that the nucleotide-biding fold is driven onto the glutamic finger. This displaces the bound GDP and leads to an 'interswitch toggle' ejecting and extending the N-terminal helix away from the protein core (Gillingham & Munro, 2007). Unlike other small G proteins of the Ras superfamily, the ARF family of proteins has a N-terminal amphipathic helix. Following removal of the initiator methionine and exposure of a glycine at position 2, a myristoyl group is attached to the N-terminus. The movement of the interswitch displaces the N-terminal amphipathic helix from the hydrophobic pocket and promotes insertion of the helix into an adjacent bilayer leading to tight membrane association (Antonny, BeraudDufour, Chardin, & Chabre, 1997). This means that ARFGEFs determine both the amount and location of the active ARF G protein.

7 | MOLECULAR PATHOLOGY OF IQSEC2 ARFGEF DOMAIN VARIANTS—DISRUPTION OF SEC7 ACTIVITY LEADING TO DIMINISHED ACTIVATION OF ARFGTPASES

The ARFGEF activity of several IQSEC2 variants has been tested in vitro, using full-length wild-type and mutant IQSEC2 expressed in cellular models in a biochemical pull-down assay using the adaptor protein Golgi-localized, ear-containing ARF-binding protein 3 (GGA3) (Kalscheuer et al., 2015; Shoubridge et al., 2010). GGAs specifically interact with active, GTP-bound ARF but do not interact with inactive ARF GTPases. This means that the degree of FLAG-tagged IQSEC2 ArfGEF activity will be reflected in the amount of GTP-bound HA-tagged ARF6 in cell lysates. After incubation with GST-tagged GGA3 coupled to glutathione beads and Western blotting, the ratio of ARF6-GTP to total ARF6 expression can be calculated, giving a measure of wild-type versus mutant IQSEC2 ARFGEF activity (Shoubridge et al., 2010). Importantly, for the Sec7 domain variants p.R758Q, p.Q801P, p.E849K, and p.R863Q, this analysis revealed that ARFGEF activity was reduced, but not abolished (Kalscheuer et al., 2015; Shoubridge et al., 2010). An important control for these experiments was the artificial p.E849K dominant negative variant, which abolishes ARFGEF activity of the IQSEC2 Sec7 domain (Beraud-Dufour et al., 1998). Molecular modeling of IQSEC2 Sec7 and PH domains has also been used to study the consequences of ARFGEF domain variants (Kalscheuer et al., 2015). The resulting model revealed that p.A789 did not interact with ARF GTPases, GDP, or Zn^2, but that the p.A789V variant was predicted to cause numerous clashes with surrounding side-chains and/or backbones of neighboring residues, so interfering with proper folding of the Sec7 domain (Kalscheuer et al., 2015).

8 | MOLECULAR PATHOLOGY OF IQSEC2 IQ-LIKE DOMAIN VARIANTS—LOSS OF Ca^{2+}-INDEPENDENT CALMODULIN BINDING?

The IQ-like motif in IQSEC2 (**IQ**TAF**R**QYRMNKN**F**) lacks the G and second basic residue of a complete motif [**F**ILV]**Q**xxx[**R**K] **G**xxx[**RK**]xx[**F**ILVWY], which would typically confer "Ca^{2+}-independent" calmodulin binding. However, this sequence conforms more closely to the consensus of IQ-like domains [**F**ILV]**Q**xxx[**R**K]Gxxxxxxx that lack a second basic residue and final anchor in the motif, or unconventional IQ motifs [**F**ILV]**Q**xxx[**R**K]**x**xxx[**RK**]xx[**F**ILVWY], which lack the glycine at position 7. IQ-like domains typically bind calmodulin in a "Ca^{2+}-dependent" manner (Munshi, Burks, Joyal, White, & Sacks, 1996). However, experimentally, the IQSEC2 IQ-like domain appears to bind calmodulin in a "Ca^{2+}-independent" manner (Myers et al., 2012), which in turn is likely to accelerate ARFGEF activity (Shoubridge et al., 2010). Currently, we know of three variants in this domain, p.R359C associated with non-syndromic intellectual disability in family AU128 (Shoubridge et al., 2010) and two variants both affecting p.A350; p.A350V associated with seizures, moderate-severe intellectual disability and ASD (Zerem, 2016), and the p.A350D variant associated with seizures, severe intellectual disability (Zhang et al., 2015). The substitution p.R359C disrupts the conserved basic residue in the IQ-like domain consensus motif (FILV)Qxxx(**R**K)xxxxxx, which leads to a decrease in GTP-bound ARF6 in the GGA pull-down assay (Shoubridge et al., 2010). Despite this, there is currently little direct proof that variants p.R359C and p.A350 variants actually disrupt IQSEC2-calmodulin interactions. However, substitution of three conserved residues within the IQ-like motif (**IQ**TAF**R** to **AA**TAF**A**, BRAG-IQ variant) has been shown to completely abrogate calmodulin binding without affecting synaptic targeting of recombinant IQSEC2 (Myers et al., 2012).

9 | MOLECULAR PATHOLOGY OF IQSEC2 PH DOMAIN VARIANTS - EFFECTS ON SYNAPTIC LOCALIZATION OR INTERACTIONS WITH MEMBRANE PHOSPHOINOSITIDES?

Several *IQSEC2* pathogenic variants also affect the PH domain, which in other GEFs implicated in intellectual disability, such as collybistin, impact binding of membrane phosphoinositides (Kalscheuer et al., 2009; Long et al., 2015; Papadopoulos, Schemm, Grubmuller, & Brose, 2015). Although the phosphoinositide binding specificity of IQSEC2 remains unknown, a related ARFGEF, IQSEC1/Brag2 binds phosphatidylinositol 4,5-bisphosphate with high affinity, which enhances intrinsic GEF activity toward Arf6 (Sakurai et al., 2011). PH domain variants affecting *IQSEC2* include both missense p.L999F (Helm et al.,

2017) and seven nonsense variants. The p.Q1033* nonsense variant is predicted to result in C-terminally truncated protein that escapes degradation via nonsense-mediated RNA decay, since the premature termination codon is very close to exon-junction. Although the Sec7 and IQ-like domains are predicted to remain intact, the PH domain would be truncated. The p.R1055* is likely degraded by NMD leading to a loss of IQSEC2, but in a heterozygous state as this patient is a female. Consistent across all cases is a phenotype of severe intellectual disability and a high proportion of seizures, although there is variability in the age of seizure onset and presence of additional features such as autism spectrum disorder, speech impairments, and deficits in ambulation. The severity of phenotype highlights the importance of an intact PH domain for IQSEC2 function, while the spectrum of features indicates that these variants may differentially impact on the function of this domain.

10 | X-LINKED INHERITANCE AND CONSEQUENCES OF GENE DOSAGE DUE TO PATHOGENIC VARIANTS IN *IQSEC2*

Although historically subcategorized as X-linked recessive and X-linked dominant, X-linked inheritance has proven to be more complex than this simple scenario. The sex determination system used in mammals is XX/XY, meaning the dosage of X-chromosomal genes is different in XX females and XY males. In humans (and other mammals), this discrepancy is circumvented by first upregulating genes on the X to match levels of expression from autosomal genes, then to balance the dosage of X chromosome genes between gender, compensation in females is achieved due to random inactivation of one of the two X chromosomes in every cell. As a consequence, when a male inherits an X-chromosome from his mother containing a deleterious gene variant, given he has no backup copy due to his XY status, the hemizygous male will reveal the full effects of the genetic mutation and will present the disease phenotype. If a female child inherits the X chromosome with a deleterious gene variant from her mother but a normal X chromosome from her father, half the cells expressing the deleterious gene will be affected on average, while the other half have normal expression. Therefore, heterozygous females will typically have a milder disease phenotype or be phenotypically normal depending upon the function of the gene involved and skewing of X-inactivation. However, some genes on the X-chromosome escape this X-inactivation resulting in differences in dosage between males and females.

The capacity of genes on the X-chromosome to be silenced, or to escape X-inactivation is not fully understood. Some genes are always inactivated or always escape inactivation across all tissues tested, with estimates of one-third of all X-chromosome genes being expressed from both active and inactive chromosome in female cells, essentially escaping inactivation (Cotton et al., 2015; Prothero, Stahl, & Carrel, 2009). This reflects ~15% of X-chromosome genes escaping inactivation, with a further ~10% displaying variable escape. Some escape genes that reside outside of the PAR have retained a Y-linked paralogue (Lahn & Page, 1997). However, escape genes that lack a functionally equivalent Y paralogue are a potential source of sex-specific differences in gene expression and thus, candidates for sex-specific phenotypes (Berletch, Yang, Xu, Carrel, & Disteche, 2011). Recent endeavors comparing four different data sets across 639 genes with X-chromosome inactivation (XCI) status calls demonstrate that XCI status is robustly called using a variety of methodologies and platforms, particularly for genes determined to be "inactivated" ($n = 462$) or "escape" ($n = 52$) across multiple tissues (Balaton, Cotton, & Brown, 2015). Although most genes display a consistent X-inactivation status across multiple tissues, there are a substantial number of genes that show variable inactivation status ($n = 47$) or give discordant status ($n = 47$) calls across different methodologies (not including those genes in the PAR regions). These variably inactivated genes are often at the boundary regions between genes that are inactive and those that escape inactivation. The chromosomal region containing *IQSEC2* is an example of this patterning of inactivation status. Escape from X-inactivation for *IQSEC2* in humans has long been the prevailing view as demonstrated by evidence measuring DNA methylation as a predictor of inactivation status across a panel of 27 tissues from 1875 females (Cotton et al., 2015). Several genes flanking *IQSEC2* at Xp11.2 are also shown to escape X-inactivation across most tissues. These genes include *KDM5C*, *SMC1A* (MIM# 300040), and *RIBC1*. In turn, these genes are flanked by *TSPYL2* (MIM# 300564) and *PHF8* (MIM# 300560), both of which are subject to inactivation in all tissues tested in these same samples (Cotton et al., 2015) (Figure 3, left hand panel). Between the escapee *RIBC1* and the inactivated *PHF8* are *HSD17B10* (MIM# 300256) and *HUWE1* (MIM# 300697) that both display variable expression across different tissues.

Despite the relatively high proportion of X-linked genes potentially escaping X-inactivation, the degree by which incomplete XCI manifests as detectable sex differences in gene expression and phenotypic traits remains poorly understood (Cotton et al., 2015; Schultz et al., 2015). Interestingly, the phenotype of female patients with de novo loss of function variants in *IQSEC2*, as outlined in this review, are often more severe than the heterozygous state would predict, particularly if *IQSEC2* does escape X-inactivation. Investigating the male–female differences in the expression of X-chromosomal genes and predicting higher female expression of genes that escape X-inactivation, large-scale expression studies from the Genotype-Tissue Expression (GTEx) project (Consortium, 2013) dataset (v6p release) based on high-coverage RNASeq data from diverse human tissues (GTEX consortia) demonstrate that *IQSEC2* is expressed at higher levels in males than females in 22 of the 29 tissues tested, including the brain cortex (Tukiainen et al., 2017) (Figure 3, right hand panel).

The genotype–phenotype relationship of patients with CNVs in this chromosomal region may provide some insights into the consequences of X-linked inheritance, as variants impacting *IQSEC2* dosage might contribute to the resulting patient phenotypes. The smallest duplicated region in patients outlined in Moey et al. (2016) contains several genes known to escape X-inactivation, including *KDM5C*, *IQSEC2*, and *SMC1A*. In silico analysis of expression data from selected gene expression omnibus series (including expression sets in brain regions) indicated that dosage of these genes, especially *IQSEC2*, is similar in males and females (Moey et al., 2016). Hence, this agrees with the recently reported sex-based differences in *IQSEC2* expression (Tukiainen et al.,

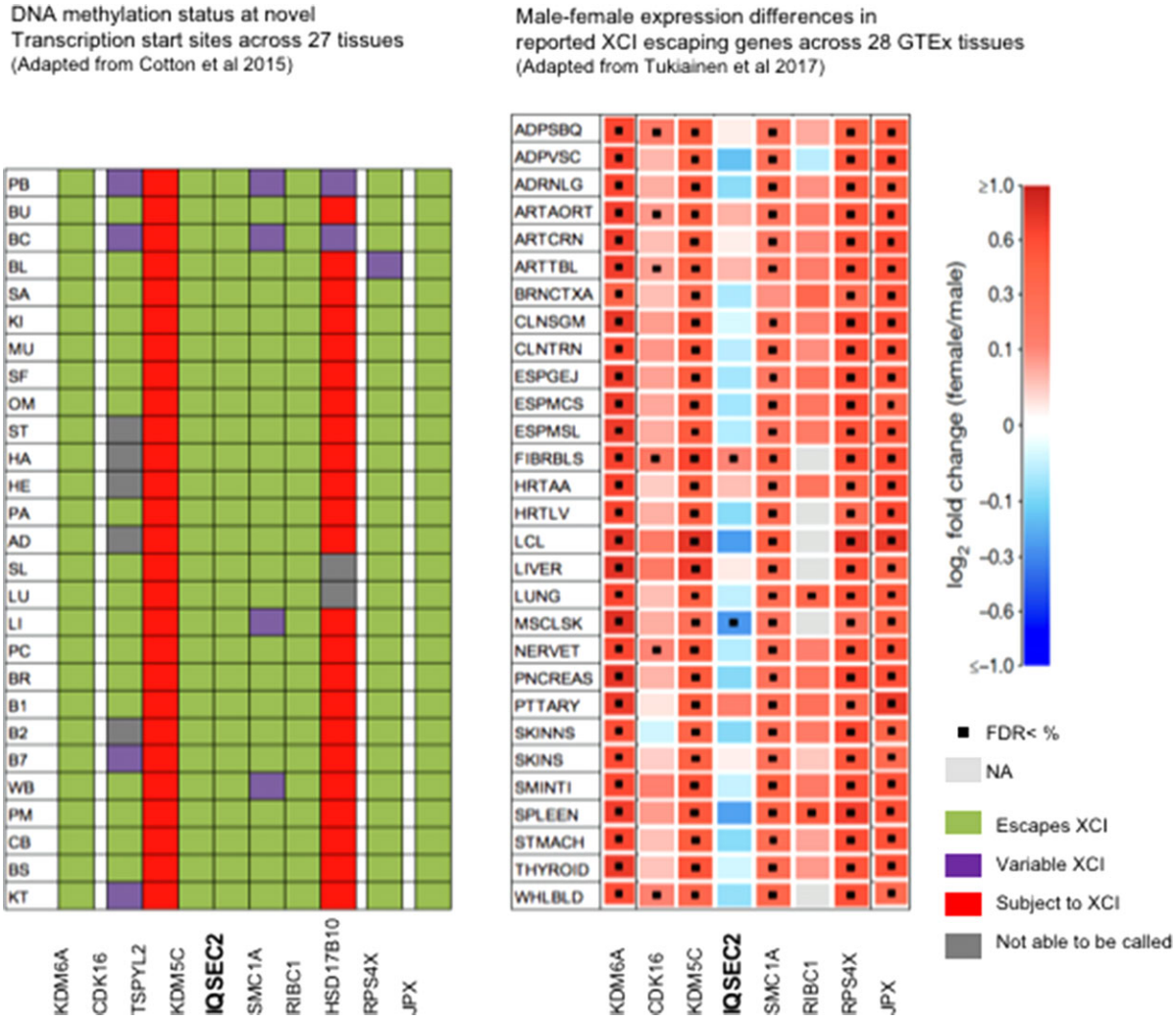

FIGURE 3 **X-inactivation status and the differences in expression of *IQSEC2* in males and females.** (a) Data in left hand panel is adapted from Cotton et al. (2015). Tissue sources are listed in the first column (see original paper for full listing of tissues and abbreviations used) and the DNA methylation status at novel transcription start sites is provided for each of the genes at Xp11.2 listed across the bottom of the panel. For each of the 27 tissues tested, escape from X-inactivation is indicated in green, genes subject to X-inactivation are highlighted in red, with variable inactivation given in purple. (b) Data in right hand panel is adapted from Tukiainen et al. (2017). The male and female expression differences across the 29 tissues analyzed as part of the GTEx resource are shown for genes reported to escape X-inactivation, with the right-hand panel focusing on the genes in the Xp11.2 region. The log fold change between expression levels in males and females is indicated based on the color map (right hand side), with expression higher in males than females indicated in blue

2017). However, if the *IQSEC2* gene is inactivated across all tissues tested, as suggested by a wide range of studies (Cotton et al., 2015), we hypothesize that the expression of *IQSEC2* in females may be regulated, by mechanisms unknown, to achieve a similar dosage to that of males. In support, the mother of an affected boy with a microduplication of Xp11.2 encompassing the *TSPYL2*, *KDM5C*, *IQSEC2*, and *SMC1A* genes was asymptomatic despite also carrying the microduplication (Moey et al., 2016), making it attractive to speculate that females may have adequate capacity to regulate *IQSEC2* expression even in the case of increased gene dosage. As males normally only have one copy of *IQSEC2*, the capacity to regulate increased dosage of this gene may be lacking. In this setting, an increased dosage of *IQSEC2* in males due to CNV gain may be pathogenic. It remains unclear how variants in the coding region of *IQSEC2* leading to loss-of-function of one allele in females have phenotypes in heterozygote females that are of similar severity when compared to that of hemizygous males. Taken together these recent analyses demonstrate that escape from XCI results in sex-bias in gene expression and lends weight to the hypothesis that incomplete XCI is a mechanism contributing to phenotypic diversity. The X-inactivation status in the brain and subsequent mechanisms contributing to dosage compensation of *IQSEC2* expression in females is poorly understood and requires investigation.

11 | MOLECULAR PATHOLOGY OF ALTERED DOSAGE OF *IQSEC2*

The biological importance in adequately regulating the dosage of *IQSEC2* expression has recently been highlighted by the findings of an

elegant study on fragile X syndrome knockout mice (Tang et al., 2015). Investigation into the proteomic profile of neocortical synaptic fractions from *Fmr1* knockout (KO) versus wild-type mice was undertaken to determine the impact of the loss of FMRI activity on de novo protein synthesis in primary cortical neurons. Interestingly, one of the most highly upregulated proteins in the synaptic fraction from fragile X mouse neurons was *Iqsec2*. These mice display phenotypic hallmarks seen in fragile X patients including impaired learning or memory, anxiety and hyperactive behavior with variable penetrance of seizures. Hence, there are clear overlaps in the phenotypes observed in patients with disturbances to *IQSEC2* expression, particularly those with duplications at Xp11.2 predicted to duplicate *IQSEC2*, i.e., potentially increase gene dosage of IQSEC2, among others (Moey et al., 2016). All the other patients reviewed as part of the present report have deficits in IQSEC2 activity or loss of IQSEC2 protein in either the hemizygous or heterozygous state. Hence, either an excess or lack of IQSEC2 activity contributes to similar phenotypic outcomes.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

ORCID

Cheryl Shoubridge http://orcid.org/0000-0002-0157-3084
Tracy Dudding-Byth http://orcid.org/0000-0002-9551-1107

REFERENCES

Allou, L., Julia, S., Amsallem, D., El Chehadeh, S., Lambert, L., Thevenon, J., … Philippe, C. (2017). Rett-like phenotypes: Expanding the genetic heterogeneity to the KCNA2 gene and first familial case of CDKL5-related disease. *Clinical Genetics*, *9*(3), 431–440.

Amor, J. C., Harrison, D. H., Kahn, R. A., & Ringe, D. (1994). Structure of the human Adp-ribosylation factor-1 complexed with Gdp. *Nature*, *372*(6507), 704–708.

Antonny, B., BeraudDufour, S., Chardin, P., & Chabre, M. (1997). N-terminal hydrophobic residues of the G-protein ADP-ribosylation factor-1 insert into membrane phospholipids upon GDP to GTP exchange. *Biochemistry*, *36*(15), 4675–4684.

Balaton, B. P., Cotton, A. M., & Brown, C. J. (2015). Derivation of consensus inactivation status for X-linked genes from genome-wide studies. *Biology of Sex Differences*, *6*, 35–46.

Beraud-Dufour, S., Robineau, S., Chardin, P., Paris, S., Chabre, M., Cherfils, J., & Antonny, B. (1998). A glutamic finger in the guanine nucleotide exchange factor ARNO displaces Mg^{2+} and the beta-phosphate to destabilize GDP on ARF1. *embo Journal*, *17*(13), 3651–3659.

Berger, S. I., Ciccone, C., Simon, K. L., Malicdan, M. C., Vilboux, T., Billington, C., … Smith, A. C. M. (2017). Exome analysis of Smith-Magenis-like syndrome cohort identifies *de novo* likely pathogenic variants. *Human Genetics*, *136*(4), 409–420.

Berletch, J. B., Yang, F., Xu, J., Carrel, L., & Disteche, C. M. (2011). Genes that escape from X inactivation. *Human Genetics*, *130*, 237–245.

Brown, J. C., Petersen, A., Zhong, L., Himelright, M. L., Murphy, J. A., Walikonis, R. S., & Gerges, N. Z. (2016). Bidirectional regulation of synaptic transmission by BRAG1/IQSEC2 and its requirement in long-term depression. *Nature Communications*, *7*, 11080.

Casanova, J. E. (2007). Regulation of Arf activation: The Sec7 family of guanine nucleotide exchange factors. *Traffic*, *8*(11), 1476–1485.

GTEx Consortium (2013). The Genotype-Tissue Expression (GTEx) project. *Nature Genetics*, *45*(6), 580–585.

Cotton, A. M., Price, E. M., Jones, M. J., Balaton, B. P., Kobor, M. S., & Brown, C. J. (2015). Landscape of DNA methylation on the X chromosome reflects CpG density, functional chromatin state and X-chromosome inactivation. *Human Molecular Genetics*, *24*(6), 1528–1539.

D'Souza-Schorey, C., & Chavrier, P. (2006). ARF proteins: Roles in membrane traffic and beyond. *Nature Reviews Molecular Cell Biology*, *7*(5), 347–358.

de Kovel, C. G., Brilstra, E. H., van Kempen, M. J., Van't Slot, R., Nijman, I. J., Afawi, Z., … Koeleman, B. P. (2016). Targeted sequencing of 351 candidate genes for epileptic encephalopathy in a large cohort of patients. *Molecular Genetics & Genomic Medicine*, *4*(5), 568–580.

de Ligt, J., Willemsen, M. H., van Bon, B. W., Kleefstra, T., Yntema, H. G., Kroes, T., … Vissers, L. E. (2012). Diagnostic exome sequencing in persons with severe intellectual disability. *New England Journal of Medicine*, *367*(20), 1921–1929.

Epi, K. C., Epilepsy Phenome/Genome, P., Allen, A. S., Berkovic, S. F., Cossette, P., Delanty, N., … Winawer, M. R. (2013). De novo mutations in epileptic encephalopathies. *Nature*, *501*(7466), 217–221.

Epi4K Consortium (2016). De novo mutations in *SLC1A2* and *CACNA1A* are important causes of epileptic encephalopathies. *American Journal of Human Genetics*, *99*(2), 287–298.

Ewans, L. J., Field, M., Zhu, Y., Turner, G., Leffler, M., Dinger, M. E., … Shoubridge, C. (2017). Gonadal mosaicism of a novel *IQSEC2* variant causing female limited intellectual disability and epilepsy. *European Journal of Human Genetics*, *25*(6), 763–767.

Fieremans, N., Van Esch, H., de Ravel, T., Van Driessche, J., Belet, S., Bauters, M., & Froyen, G. (2015). Microdeletion of the escape genes *KDM5C* and *IQSEC2* in a girl with severe intellectual disability and autistic features. *European Journal of Medical Genetics*, *58*(5), 324–327.

Gandomi, S. K., Farwell Gonzalez, K. D., Parra, M., Shahmirzadi, L., Mancuso, J., Pichurin, P., … Tang, S. (2014). Diagnostic exome sequencing identifies two novel *IQSEC2* mutations associated with X-linked intellectual disability with seizures: Implications for genetic counseling and clinical diagnosis. *Journal of Genetic Counseling*, *23*(3), 289–298.

Gilissen, C., Hehir-Kwa, J. Y., Thung, D. T., van de Vorst, M., van Bon, B. W. M., Willemsen, M. H., … Veltman, J. A. (2014). Genome sequencing identifies major causes of severe intellectual disability. *Nature*, *511*(7509), 344–347.

Gillingham, A. K., & Munro, S. (2007). The small G proteins of the Arf family and their regulators. *Annual Review of Cell and Developmental Biology*, *23*, 579–611.

Goldberg, J. (1998). Structural basis for activation of ARF GTPase: Mechanisms of guanine nucleotide exchange and GTP-myristoyl switching. *Cell*, *95*(2), 237–248.

Hamdan, F. F., Myers, C. T., Cossette, P., Lemay, P., Spiegelman, D., Laporte, A. D., … Michaud, J. L. (2017). High rate of recurrent de novo mutations in developmental and epileptic encephalopathies. *American Journal of Human Genetics*, *101*, 664–685.

Helbig, K. L., Farwell Hagman, K. D., Shinde, D. N., Mroske, C., Powis, Z., Li, S., … Helbig, I. (2016). Diagnostic exome sequencing provides a molecular diagnosis for a significant proportion of patients with epilepsy. *Genetics in Medicine*, *18*(9), 898–905.

Helm, B. M., Powis, Z., Prada, C. E., Casasbuenas-Alarcon, O. L., Balmakund, T., Schaefer, G. B., … Schrier Vergano, S. A. (2017). The role of IQSEC2 in syndromic intellectual disability: Narrowing the diagnostic odyssey. *American Journal of Medical Genetics. Part A*, *173*(10), 2814–2820.

Hinze, S. J., Jackson, M. R., Lie, S., Jolly, L., Field, M., Barry, S. C., … Shoubridge, C. (2017). Incorrect dosage of *IQSEC2*, a known intellectual disability and epilepsy gene, disrupts dendritic spine morphogenesis. *Translational Psychiatry*, *7*(5), e1110.

Hu, H., Haas, S. A., Chelly, J., Van Esch, H., Raynaud, M., de Brouwer, A. P., … Kalscheuer, V. M. (2016). X-exome sequencing of 405 unresolved families identifies seven novel intellectual disability genes. *Molecular Psychiatry*, *21*(1), 133–148.

Kahn, R. A., Cherfils, J., Elias, M., Lovering, R. C., Munro, S., & Schurmann, A. (2006). Nomenclature for the human Arf family of GTP-binding proteins: ARF, ARL, and SAR proteins. *Journal of Cell Biology*, *172*(5), 645–650.

Kalscheuer, V. M., James, V. M., Himelright, M. L., Long, P., Oegema, R., Jensen, C., … Harvey, R. J. (2015). Novel missense mutation A789V in *IQSEC2* underlies X-linked intellectual disability in the MRX78 family. *Frontiers in Molecular Neuroscience*, *8*, 85.

Kalscheuer, V. M., Musante, L., Fang, C., Hoffmann, K., Fuchs, C., Carta, E., … Harvey, R. J. (2009). A balanced chromosomal translocation disrupting *ARHGEF9* is associated with epilepsy, anxiety, aggression, and mental retardation. *Human Mutation*, *30*(1), 61–68.

Lahn, B. T., & Page, D. C. (1997). Functional coherence of the human Y chromosome. *Science*, *278*, 675–680

Long, P., May, M. M., James, V. M., Granno, S., Johnson, J. P., Tarpey, P., … Harvey, R. J. (2015). Missense mutation R338W in *ARHGEF9* in a family with X-linked Intellectual disability with variable macrocephaly and macro-orchidism. *Frontiers in Molecular Neuroscience*, *8*, 83.

Madrigal, I., Alvarez-Mora, M. I., Rosell, J., Rodriguez-Revenga, L., Karlberg, O., Sauer, S., … Mila, M. (2016). A novel splicing mutation in the IQSEC2 gene that modulates the phenotype severity in a family with intellectual disability. *European Journal of Human Genetics*, *24*(8), 1117–1123.

Menetrey, J., Macia, E., Pasqualato, S., Franco, M., & Cherfils, J. (2000). Structure of Arf6-GDP suggests a basis for guanine nucleotide exchange factors specificity. *Nature Structural Biology*, *7*(6), 466–469.

Mignot, C., McMahon, A. C., Bar, C., Campeau, P. M., Davidson, C., Buratti, J., … Depienne, C. (2018). IQSEC2-related encephalopathy in males and females: A comparative study including 37 novel patients. *Genetics in Medicine*. https://doi.org/10.1038/s41436-018-0268-1

Moey, C., Hinze, S. J., Brueton, L., Morton, J., McMullan, D. J., Kamien, B., … Shoubridge, C. (2016). Xp11.2 microduplications including *IQSEC2*, *TSPYL2* and *KDM5C* genes in patients with neurodevelopmental disorders. *European Journal of Human Genetics*, *24*(3), 373–380.

Morleo, M., & Franco, B. (2008). Dosage compensation of the mammalian X chromosome influences the phenotypic variability of X-linked dominant male-lethal disorders. *Journal of Medical Genetics*, *45*(7), 401–408.

Munshi, H. G., Burks, D. J., Joyal, J. L., White, M. F., & Sacks, D. B. (1996). Ca^{2+} regulates calmodulin binding to IQ motifs in IRS-1. *Biochemistry*, *35*(49), 15883–15889.

Murphy, J. A., Jensen, O. N., & Walikonis, R. S. (2006). BRAG1, a Sec7 domain-containing protein, is a component of the postsynaptic density of excitatory synapses. *Brain Research*, *1120*(1), 35–45.

Myers, K. R., Wang, G., Sheng, Y., Conger, K. K., Casanova, J. E., & Zhu, J. J. (2012). Arf6-GEF BRAG1 regulates JNK-mediated synaptic removal of GluA1-containing AMPA receptors: A new mechanism for nonsyndromic X-linked mental disorder. *Journal of Neuroscience*, *32*(34), 11716–11726.

Olson, H. E., Tambunan, D., LaCoursiere, C., Goldenberg, M., Pinsky, R., Martin, E., … Poduri, A. (2015). Mutations in epilepsy and intellectual disability genes in patients with features of Rett syndrome. *American Journal of Medical Genetics. Part A*, *167A*(9), 2017–2025.

Papadopoulos, T., Schemm, R., Grubmuller, H., & Brose, N. (2015). Lipid binding defects and perturbed synaptogenic activity of a Collybistin R290H mutant that causes epilepsy and intellectual disability. *Journal of Biological Chemistry*, *290*(13), 8256–8270.

Parrini, E., Marini, C., Mei, D., Galuppi, A., Cellini, E., Pucatti, D., … Guerrini, R. (2017). Diagnostic targeted resequencing in 349 patients with drug-resistant pediatric epilepsies identifies causative mutations in 30 different genes. *Human Mutation*, *38*(2), 216–225.

Pasqualato, S., Renault, L., & Cherfils, J. (2002). Arf, Arl, Arp and Sar proteins: A family of GTP-binding proteins with a structural device for 'front-back' communication. *EMBO Reports*, *3*(11), 1035–1041.

Piton, A., Gauthier, J., Hamdan, F. F., Lafrenière, R. G., Yang, Y., Henrion, E., … Rouleau, G. A. (2011). Systematic resequencing of X-chromosome synaptic genes in autism spectrum disorder and schizophrenia. *Molecular Psychiatry*, *16*(8), 867–880.

Prothero, K. E., Stahl, J. M., & Carrel, L. (2009). Dosage compensation and gene expression on the mammalian X chromosome: One plus one does not always equal two. *Chromosome Research*, *17*(5), 637–648.

Rauch, A., Wieczorek, D., Graf, E., Wieland, T., Endele, S., Schwarzmayr, T., … Strom, T. M. (2012). Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: An exome sequencing study. *Lancet*, *380*(9854), 1674–1682.

Redin, C., Gerard, B., Lauer, J., Herenger, Y., Muller, J., Quartier, A., … Piton, A. (2014). Efficient strategy for the molecular diagnosis of intellectual disability using targeted high-throughput sequencing. *Journal of Medical Genetics*, *51*(11), 724–736.

Renault, L., Christova, P., Guibert, B., Pasqualato, S., & Cherfils, J. (2002). Mechanism of domain closure of Sec7 domains and role in BFA sensitivity. *Biochemistry*, *41*(11), 3605–3612.

Roy, N. S., Yohe, M. E., Randazzo, P. A., & Gruschus, J. M. (2016). Allosteric properties of PH domains in Arf regulatory proteins. *Cellular Logistics*, *6*(2), e1181700.

Sajan, S. A., Jhangiani, S. N., Muzny, D. M., Gibbs, R. A., Lupski, J. R., Glaze, D. G., … Neul, J. L. (2017). Enrichment of mutations in chromatin regulators in people with Rett syndrome lacking mutations in *MECP2*. *Genetics in Medicine*, *19*(1), 13–19.

Sakagami, H., Sanda, M., Fukaya, M., Miyazaki, T., Sukegawa, J., Yanagisawa, T., … Kondo, H. (2008). IQ-ArfGEF/BRAG1 is a guanine nucleotide exchange factor for Arf6 that interacts with PSD-95 at postsynaptic density of excitatory synapses. *Neuroscience Research*, *60*(2), 199–212.

Sakurai, A., Jian, X. Y., Lee, C. J., Manavski, Y., Chavakis, E., Donaldson, J., … Gutkind, J. S. (2011). Phosphatidylinositol-4-phosphate 5-Kinase and GEP100/Brag2 Protein Mediate Antiangiogenic Signaling by Semaphorin 3E-Plexin-D1 through Arf6 Protein. *Journal of Biological Chemistry*, *286*(39), 34335–34345.

Sanda, M., Kamata, A., Katsumata, O., Fukunaga, K., Watanabe, M., Kondo, H., & Sakagami, H. (2009). The postsynaptic density protein, IQ-ArfGEF/BRAG1, can interact with IRSp53 through its proline-rich sequence. *Brain Research*, *1251*, 7–15.

Schultz, M. D., He, Y. P., Whitaker, J. W., Hariharan, M., Mukamel, E. A., Leung, D., … Ecker, J. R. (2015). Human body epigenome maps reveal noncanonical DNA methylation variation. *Nature*, *523*(7559), 212–U189.

Shoubridge, C., Tarpey, P. S., Abidi, F., Ramsden, S. L., Rujirabanjerd, S., Murphy, J. A., … Gécz, J. (2010). Mutations in the guanine nucleotide exchange factor gene IQSEC2 cause nonsyndromic intellectual disability. *Nature Genetics*, *42*(6), 486–488.

Tang, B., Wang, T. T., Wan, H. D., Han, L., Qin, X. Y., Zhang, Y. Y., … Liao, L. (2015). Fmr1 deficiency promotes age-dependent alterations in the cortical synaptic proteome. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(34), E4697–E4706.

Tarpey, P. S., Smith, R., Pleasance, E., Whibley, A., Edkins, S., Hardy, C., … Stratton, M. R. (2009). A systematic, large-scale resequencing screen of X-chromosome coding exons in mental retardation. *Nature Genetics*, *41*(5), 535–543.

Tran Mau-Them, F., Willems, M., Albrecht, B., Sanchez, E., Puechberty, J., Endele, S., … Genevieve, D. (2014). Expanding the phenotype of *IQSEC2* mutations: Truncating mutations in severe intellectual disability. *European Journal of Human Genetics*, *22*(2), 289–292.

Tukiainen, T., Villani, A. C., Yen, A., Rivas, M. A., Marshall, J. L., Satija, R., … MacArthur, D. G. (2017). Landscape of X chromosome inactivation across human tissues. *Nature*, *550*(7695), 244–248.

Tzschach, A., Grasshoff, U., Beck-Woedl, S., Dufke, C., Bauer, C., Kehrer, M., … Bauer, P. (2015). Next-generation sequencing in X-linked intellectual disability. *European Journal of Human Genetics*, *23*(11), 1513–1518.

Um, J. W. (2017). Synaptic functions of the IQSEC family of ADP-ribosylation factor guanine nucleotide exchange factors. *Neuroscience Research*, *116*, 54–59.

Vetter, I. R., & Wittinghofer, A. (2001). Signal transduction - The guanine nucleotide-binding switch in three dimensions. *Science*, *294*(5545), 1299–1304.

Zerem, A., Haginoya, K., Lev, D., Blumkin, L., Kivity, S., Linder, I., … Lerman-Sagie, T. (2016). The molecular and phenotypic spectrum of IQSEC2-related epilepsy. *Epilepsia*, *57*(11), 1858–1869.

Zhang, Y., Kong, W., Gao, Y., Liu, X., Gao, K., Xie, H., … Jiang, Y. (2015). Gene Mutation Analysis in 253 Chinese Children with Unexplained Epilepsy and Intellectual/Developmental Disabilities. *PLoS One*, *10*(11), e0141782.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Shoubridge C, Harvey RJ, Dudding-Byth T. *IQSEC2* mutation update and review of the female-specific phenotype spectrum including intellectual disability and epilepsy. *Human Mutation*. 2019;40:5–24. https://doi.org/10.1002/humu.23670