SHORT REPORT

Gonadal mosaicism of a novel *IQSEC2* variant causing female limited intellectual disability and epilepsy

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We report a family with four girls with moderate to severe intellectual disability and epilepsy. Two girls showed regression in adolescence and died of presumed sudden unexpected death in epilepsy at 16 and 22 years. Whole exome sequencing identified a truncating pathogenic variant in *IQSEC2* at NM_001111125.2: c.2679_2680insA, p.(D894fs*10), a recently identified cause of epileptic encephalopathy in females (MIM 300522). The *IQSEC2* variant was identified in both surviving affected sisters but in neither parent. We describe the phenotypic spectrum associated with *IQSEC2* variants, highlighting how *IQSEC2* is adding to a growing list of X-linked genes that have a female-specific phenotype typically associated with *de novo* mutations. This report illustrates the need for careful review of all whole exome data, incorporating all possible modes of inheritance including that suggested by the family history.

European Journal of Human Genetics (2017) 25, 763–767; doi:10.1038/ejhg.2017.29; published online 15 March 2017

INTRODUCTION

The first variants that affects function in the IQ motif and Sec7 domain 2 (*IQSEC2*) gene [NM_001111125.2] (MIM 300522) were described in four separate families with X-linked intellectual disability (XLID).¹ Since our first report describing familial cases, high throughput sequencing in intellectual disability or epilepsy cohorts has identified *de novo* loss-of-function *IQSEC2* variants in males and increasingly in females.^{2–11} *IQSEC2* catalyzes exchange of GDP for GTP in a number of the ARF superfamily of proteins. It has an essential role in modulating the cytoskeleton and vesicle transport at the post-synaptic density and hence is a crucial modifier of synaptic plasticity. All variants were missense and clustered around the sec7 and IQ-like domains, resulting in reduced enzymatic activity.¹ Complete abolition of enzymatic activity leads to a more severe phenotype including epileptic encephalopathy (EE) in both males and females. A recent review by Zarem and colleagues¹² identified 18 individuals in the literature with *IQSEC2* variants and seizures; five of the six females described had a *de novo* loss-of-function variant.^{3,5,7} *IQSEC2* adds to a growing list of genes on the X chromosome, such as *USP9X* and *PHF6*, that have distinct phenotypes in males and females depending on the functional severity of the variant.^{13,14}

MATERIALS AND METHODS

WES identification and molecular analysis

This work was undertaken as part of a study on whole exome sequencing (WES) application to diagnosis in Mendelian disorders at Sydney Children's Hospital and the Kinghorn Centre for Clinical

Genomics, approved by the local ethics committee (HREC ref no 13/094). High-quality DNA was extracted from peripheral blood in EDTA. WES was performed on two of the five affected sisters for genetic diagnosis using a NimbleGen VCRome rapid capture expanded exome kit, with libraries analysed on an Illumina HiSeq2500 instrument. Bioinformatics filtering steps and scientific methods are described in Supplementary Methods.

RESULTS

Phenotype

We report a family of six girls born to non-consanguineous Caucasian parents (Figure 1 and Table 1). Four girls had a similar course with developmental delay followed by onset of seizures at 2–5 years of age. Focal seizures were most prominent with seizure triggers including fever, immunisation and the perimenstrual period. They had intellectual disability, which ranged from moderate to severe and aggressive behaviour when young with some autistic features. Two girls showed regression in adolescence, both dying of presumed sudden unexpected death in epilepsy (SUDEP) at 16 and 22 years. The surviving two affected sisters live at home in the care of their parents and are now in their early 40's and have infrequent seizures that often occur around the time of menstruation. II-2 is 159 cm in height (25th centile) with OFC of 57cm (95th centile; Centile based on CDC growth charts). II-5 is 147cm tall (1st centile) with OFC of 55cm (30th centile). There were no dysmorphic features as adults (Figure 1a), and childhood photographs show no evidence of dysmorphic features.

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Received 19 October 2016; revised 23 January 2017; accepted 7 February 2017; published online 15 March 2017

Figure 1 Identification of a c.2679_2680insA (NM_001111125.2) variant resulting in p.(D894fs*10) (NP_001104595) in *IQSEC2*. (a) Pedigree of family. Facial photos of II-2 and II-5 shown above the pedigree. Open symbols represent unaffected individuals, filled grey circle represents a female of borderline intellect to mild intellectual disability with ASD, while filled black circles represent females with moderate intellectual disability and epilepsy. Normal (N) and mutant (M) alleles shown for individuals tested. (b) DNA sequence electropherograms for the chrX:g.53276220_53276221insT (GRCh37/hg19 assembly); c.2679_2680insA mutation in exon 8 of 15 of *IQSEC2*. (c) Predicted impact of the novel variants in *IQSEC2*. The exon-intron structure of the longest isoform of *IQSEC2* gene [NM_001111125.2] with 15 exons, the ATG, open reading frame and stop codon positions in black and 5' and 3' untranslated regions in light grey. The predicted protein structure (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), corresponding amino acids listed below each domain. The variant c.2679_2680insA causes a frame-shift at the site of insertion with the premature stop codon 29 nucleotides subsequent of the insertion, 41 nucleotides from the exon 8 and 9 junction. This transcript may escape nonsense mediated decay. In addition to a 9 amino acid nonsense peptide encoded prior to the PTC, the C-terminally truncated protein would lack a portion of the Sec7 domain, the PH domain and the PDZ binding domain.

Table 1 Clinical features and investigations of family with novel insertion variant in *IQSEC2*

Clinical Features	II-1	II-2	II-3	II-4	II-5
<i>Mutation</i>					
Status – Affected female (AF), current age (yrs) p.D894fsX10 in <i>IQSEC2</i>	AF Deceased aged 22 yrs Not tested	AF 49 yrs Yes	AF Deceased aged 16 yrs Not tested	AF 46 yrs No (<i>IQSEC2</i> WT)	AF 44 yrs Yes
<i>Development</i>					
Early development	Mild delay	Developmental slowing after second immunization (pertussis, diphtheria, tetanus) ~4 months	Severe delay (not walking at 33/12)	Mild delay (walking 18/12; single words 24/12)	Minor delay
Language development	Intellectual disability	Some language – better receptive language	Non-verbal	Mild intellectual disability	Non-verbal
Schooling	Special school, life skills	Special school, life skills	Home schooling	Home schooling, modified curriculum	Home schooling, modified curriculum
Independent living and social skills	Dependent	Dependent, some self care skills	Dependent	Semi independent, works in supported environment	Dependent, some self care skills
Deceased	22 years: found dead in bed Presumed SUDEP (no autopsy)	No	16 years: presumed SUDEP	No	No
<i>Epilepsy</i>					
Age of onset	Yes	Yes	Yes	No	Yes
Seizure types	5 years Focal impaired awareness seizures, tonic-clonic seizures ~3 / wk	2 years Febrile tonic-clonic after measles. Perimenstrual absence and focal seizures	2 years Focal motor seizure	Nil	2 years Focal impaired awareness seizures Perimenstrual tonic-clonic
Anti-epileptic therapy	Phenytoin, carbamazepine; uncontrolled	Phenytoin and carbamazepine	Phenytoin	Nil	Phenytoin and carbamazepine
<i>Other features</i>					
Behaviour	Autistic traits, aggressive behaviour in early childhood	Early childhood; screaming, ADHD		Autistic features	
Regression	16 yrs: anorexia, food refusal, social withdrawal		Adolescent social withdrawal, concomitant deteriorating condition	ASD	
Neurological examination (childhood)	Limited coordination, stiff movement	Nil	Nil	Nil	Nil
Other features	Mild scoliosis		EEG: continual left hemisphere discharges and slowing	True knot in umbilical cord Shorter stature than siblings	Mild scoliosis
Normal Investigations		CDKL5 coding region, Epileptic Encephalopathy gene panel	Karyotype and urine metabolic screen	CDKL5 coding regions; X-tiling array, genome-wide array (0.2 Mb res)	PCDH19 and CDKL5 coding regions; MECP2 MLPA / sequencing; fatty acid, urine metabolic screen

Abbreviation: SUDEP, Sudden unexpected death in epilepsy.

The fifth daughter, II-4, had autistic features and borderline to mild intellectual disability, without seizures. She had perinatal complications with a true knot in her cord at birth. The sixth daughter, II-6, was unaffected and of normal intellect; she worked in a senior managerial role. There was one single early miscarriage (sex unknown) and no male children. Both parents were health professionals and have no history of seizures or cognitive problems.

Molecular analysis of the *IQSEC2* variant

WES analysis identified a heterozygous novel single base pair insertion shared by both sisters at genomic position chrX:g.53276220_53276221insT (GRCh37/hg19 assembly; Supplementary Figure 1). This variant in exon 8 of the NM_001111125.2 isoform inserts a single nucleotide at c.2679_2680insA, p.(D894fs*10) (NP_001104595) in *IQSEC2*. Variant has been submitted to the gene variant database at www.LOVD.nl/IQSEC2 (patient ID #00081545; DB-ID #000015). Exons are numbered like in NG_021296.1. Sanger sequencing confirmed the presence of this variant in both affected sisters II-2 and II-5 (Figures 1a and b; Supplementary Figure 2). The *IQSEC2* single base pair insertion was absent in the father (I-1), mother (I-2) and their unaffected (II-6) and phenotypically distinct (II-4) sisters. Identification of parental origin of the mosaicism was not undertaken at the request of the family.

The insertion of this single nucleotide is predicted to cause a frameshift with a premature stop codon 29 nucleotides downstream (Figure 1c). As expression of *IQSEC2* is negligible in lymphoblast cells and fibroblasts, we have been unable to determine if the mutant allele escapes nonsense mediated decay (NMD).

DISCUSSION

IQSEC2 is a gene on the X-chromosome initially implicated in XLID through identification of variants in affected males in large X-linked families.¹ Subsequent population-based screening approaches in intellectual disability and epilepsy cohorts using next generation sequencing (NGS) strategies have identified variants predicted to affect function in *IQSEC2* in both sexes. This study identified a novel frameshift variant in *IQSEC2*, NM_001111125.2: c.2679_2680insA, p.(D894fs*10) in affected females that was absent in DNA extracted from both blood and saliva samples in their unaffected parents. Parental gonadal mosaicism is therefore the most likely mechanism, with the parent of origin not clarified. Gonadal mosaicism as a cause of sibling recurrence of genetic disorders is an increasingly recognised molecular mechanism, with studies suggesting as many as 3.8–10% of apparently *de novo* variants are mosaic in the parental germline.^{15,16}

The *IQSEC2* variant identified in this family truncates the protein within the Sec7 domain, but distal to the critical residue for enzymic activity at p.E849. The premature stop codon is encoded just 41 nucleotides from the exon 8 and 9 junction, less than the required 50 nucleotides required to trigger NMD. As such, we cannot rule out that the RNA may escape NMD to some extent. Despite this, even if a C-terminal truncated protein was produced and potentially retain residual Sec7 enzyme activity, it would lack both the PH and PDZ domains. The PH domain is predicted to mediate membrane localisation and play a regulatory role in binding to phosphoinositides, with the type 1 PDZ domain at the C-terminal end of the *IQSEC* protein required for interaction with the PSD complex.¹⁷ Removal of last 74 amino acids of the protein results in a dramatic mis-localisation of the truncated protein to the dendritic shaft of neurons instead of

Table 2 Summary of *IQSEC2* variants reported in affected males and females

Mutation cDNA	Ex	Mutation Protein AA	Domain affected	Mutation type	Phenotype	Family	Inheritance	Sex	GRCh37/hg19 coordinate	Reference
c.273_282del	1	p.N91Kfs*112	–	nonsense	Rett like	P7	De novo	F	53350040-53350049	6
c.804delC	3	p.Y269Tfs*3	–	nonsense	Seizures	48	De novo	F	53285177	9
c.928G>T	3	p.E310*	–	nonsense	FE, mild-mod ID	P16	De novo	F	53285053	12
c.1049C>T	4	p.A350V	IQ-like	missense	EE, mod-severe ID	P1	De novo	M	53284064	12
c.1075C>T	4	p.R359C	IQ-like	missense	NS-XLID	AU128	Familial	M	53284038	1
c.1618C>T	5	p.Q540*	–	nonsense	EE, DD	P14	De novo	M	53280140	12
c.1688G>A	5	p.R563Q	–	missense	ASD	S0090	Familial	M	53280070	19(supp)
c.2052_2053delCG	5	p.C684*	–	nonsense	DD, Seizures	P1	De novo	M	53279705-53279706	5
					Seizures	47	De novo	F		9
c.2203C>T	5	p.Q735*	–	nonsense	SGE	T17563	De novo	F	53279555	11
c.2273G>A	5	p.R758Q	Sec7	missense	NS-XLID	US166	Familial	M	53279485	1
c.2366C>T	6	p.A789V	Sec7	missense	Severe-ID, Seizures, Ag Mild-ID	MRX78	Familial	M	53277996	8
c.2402 A>C	6	p.Q801P	Sec7	missense	NS-XLID	MRX18	Familial	M	53277960	1
c.2563C>T	7	p.R855*	Sec7	nonsense	NS-XLID		De novo	M	53277315	2
c.2582G>C	7	p.S861T	Sec7	splicing	DD, Seizures	P2	De novo	M	53277296	4
					Seizures	46	De novo	F		9
c.2587C>T	8	p.R863W	Sec7	missense	NS-XLID	MRX1	Familial	M	53276313	1
c.2662dup	8	p.I888Nfs*16	Sec7	nonsense	XLID	P7	De novo	M	53276237	7
c.2679_2680insA	8	p.(D894fs*10)	Sec7	nonsense	ID and epilepsy	K2	Familial	F	53276220	This report
c.2776C>T	9	p.R926*	Sec7	nonsense	Rett like	P3		F	3272627	10
c.3097C>T	11	p.Q1033*	PH	nonsense	Severe ID, Seizures	APN-68	De novo	M	53268395	5
c.3163C>T	12	p.R1055*	PH	nonsense	Severe ID,	Fam 19		F	53267441	7
c.3322C>T	13	p.Q1108*	–	nonsense	LGS	KO	De novo	F	53265633	3

Abbreviations: Ag, Aggression; ASD-F, autistic features; DevR, developmental regression; DD, Developmental delay; EE, Epileptic encephalopathy; FE, Focal epilepsy; ID, intellectual disability; LGS, Lennox-Gastaut syndrome; NS-XLID, non-syndromic X-linked intellectual disability; SGE, symptomatic generalised epilepsy. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence for the *IQSEC2* gene [GenBank: NM_001111125.2].

the usual location within the dendritic spines.¹⁸ Even if the C-terminal protein was to escape NMD to some degree, the impact on the functional capacity of the mutant *IQSEC2* protein is predicted to be consistent with a loss-of-function and subsequent severe phenotype in affected females.

We assessed all the reported missense and nonsense variants associated with disease reported in *IQSEC2*, listing them as they occur across the length of the coding region^{1–11,19} (Table 2), and several notable correlations were identified. The missense variants that either impact the Sec7 domain directly or interfere with Sec7 activity are all inherited in a familial manner (pale grey in Table 2). As highlighted by the recent report in the MRX78 family, these families present with affected males having a more severe phenotype than affected females.⁹ The remaining cases (dark grey in Table 2) present with a spectrum of seizure and behavioural phenotypes due to *de novo* nonsense variants or variants that lead to splicing defects.

Affected females with *de novo* variants present with epileptic encephalopathy including Rett-like phenotypes and Lennox-Gastaut syndrome. Our affected sisters presented with epilepsy and intellectual disability. They may have had an epileptic encephalopathy (EE) but there was only scant EEG data to show that developmental slowing in infancy or the adolescent regression in two sisters had an epileptic basis. Interestingly these two sisters died of presumed SUDEP in adolescence and young adult life. This is the first report of SUDEP in *IQSEC2* diseases suggesting that *IQSEC2* adds to the number of EE genes that may increase risk for SUDEP.²⁰

This gene region is also interesting from an epigenetic perspective. *IQSEC2* escapes X-inactivation in females.²¹ Genes that escape X-inactivation are potential candidates for dosage-mediated phenotypic disruptions. This has been shown recently for microduplications in Xp11.2 involving the *IQSEC2* gene.²² Even though X inactivation is not apparent at this loci, expression of *IQSEC2* is similar in males and females.²² We speculate that if a female has a heterozygous variant in *IQSEC2* that results in a significant loss-of-function of *IQSEC2* activity, the as-yet unknown mechanism that limits the gene dosage of *IQSEC2* to mirror that in males does not allow for compensation for the loss of *IQSEC2* function in females. The *IQSEC2* region contributes to a growing body of evidence that the extent of escape from X-inactivation may be underappreciated as a substantial number of genes are subject to some degree of escape.²³

The phenotypic spectrum associated with these cases demonstrates that *IQSEC2* should be considered as an X-linked gene extending to a female-specific phenotype, including intellectual disability, epilepsy and SUDEP. In addition we suggest careful analysis of NGS data with consideration of all modes of inheritance, even those that seem unlikely based on family history.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank the patients, their families and physicians for their participation in this study, in particular Dr Michael Partington for his work with this family over many years. We would like to thank Kevin Ying, Jiang Tao, Paula Morris and Kerith-Rae Dias from the Kinghorn Centre for Clinical Genomics for sequencing these patients. Whole exome sequencing and salaries for TR and

MED were funded through a donation by the Kinghorn Foundation to the Garvan Institute of Medical Research. MJC is supported by Cancer Institute NSW (13/ECE/1–46). CS is supported by the Australian Research Council (Future Fellowship FT120100086).

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Supplementary Information accompanies this paper on European Journal of Human Genetics website (<http://www.nature.com/ejhg>)