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Deep phenotyping of fourteen new patients with *IQSEC2* variants, including monozygotic twins of discordant phenotype.

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DDD statement

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Abstract

Whole exome sequencing has established *IQSEC2* as a neurodevelopmental disability gene. The *IQSEC2* variant phenotype includes developmental delay, intellectual disability, epilepsy, hypotonia, autism, developmental regression, microcephaly and stereotypies but is yet to be fully described. Presented here, are 14 new patients with *IQSEC2* variants. In addition to the established features, we observed: gait ataxia in 7/9 (77.8%), drooling in 9/14 (64.2%), early feeding difficulties in 7/14 (50%), structural brain abnormalities, in 6/13 (46.2%), brachycephaly in 5/14 (35.7%), and scoliosis and paroxysms of laughter, each in 4/14 (28.6%). We suggest that these are features of the *IQSEC2*-related disorder. Gastrostomy requirement, plagiocephaly, strabismus and cortical blindness, each seen in 2/14 (14.3%) may also be associated. Shared facial features were noted in 8/14 patients and shared hair patterning was identified in 4/14 patients.

This study further delineates the *IQSEC2* phenotypic spectrum and supports the notion of an emerging *IQSEC2* syndrome. We draw parallels between the *IQSEC2*-related disorder and the Angelman- / Rett- / Pitt-Hopkins syndrome group of conditions and recommend the addition of *IQSEC2* to epilepsy- and developmental delay gene panels. We observed discordant phenotypes in monozygotic twins and apparent gonadal mosaicism, which has implications for recurrence risk counselling in the *IQSEC2*-related disorder.

Introduction

The IQ motif and Sec7 domain 2 (*IQSEC2*) gene (OMIM #300522), located at chromosome Xp11.22, has recently been identified as an important neurodevelopment gene by next generation sequencing studies. 85 cases or families with *IQSEC2* variants have been previously described in the medical literature, however, detailed case studies of only about 30 patients with *IQSEC2* variants have so far been reported and the phenotype has not yet been fully delineated.

IQSEC2 was first ascertained as a gene of neurodevelopment in 2008, when a girl with infantile spasms, profound developmental delay and severe intellectual disability was found to have a chromosomal translocation with a break point disrupting the first intron of *IQSEC2*[1]. Murine studies demonstrated that the IQSEC2 protein (also known as BRAG1) is expressed in the central nervous system in early development[1]. IQSEC2 is one of the most abundant proteins in the post synaptic density of glutaminergic neurons - more so than N-methyl-D-aspartate (NMDA) receptors or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, implying that it is of crucial importance in post synaptic transmission[2].

The established features of patients with *IQSEC2* variants are global developmental delay, intellectual disability, epilepsy, microcephaly, hypotonia, language regression, social communication deficits and stereotypic hand movements[3]. In one study, 7/9 patients with *IQSEC2* variants who underwent Magnetic Resonance Imaging (MRI) of the brain demonstrated abnormal -but non-specific- MRI appearances[4].

Much of the literature on humans with *IQSEC2* variants takes the form of sequencing and reporting variants in patients with epilepsy. In this, the joint second largest *IQSEC2* phenotyping study to date, we present 14 new patients with *IQSEC2* variants, identified through the Deciphering Developmental Disorders (DDD) study and further delineate the features of the *IQSEC2*-related disorder.

Materials and methods

Patients with a wide range of neurodevelopmental problems were recruited to the DDD study[5], with trio whole exome sequencing performed as previously described[6]. All patients had had an uninformative chromosomal microarray prior to recruitment. *IQSEC2* (NM_001111125.2) variants identified were validated by targeted Sanger sequencing.

An application to the DDD study for a complimentary analysis project was successfully made, allowing access to anonymised details of patients with *IQSEC2* variants identified through DDD on the Decipher website[7]. Clinicians of selected patients were contacted to invite patients and their families to be recruited. Excluded from the study were: patients with an additional proven genetic diagnosis, those with a chromosomal anomaly and those in whom *IQSEC2* variants were of unknown inheritance or unknown clinical significance, with the exception of two cases, where the clinician had a high degree of suspicion that the variant was causative.

The clinical significance of the variants identified was interpreted according to the guidelines set out by the American College of Medical Genetics[8].

Written consent for patients' clinical details and photographs to be used in journal publication was provided by the parents of the patients.

Results

Fourteen patients with variants in the *IQSEC2* gene were recruited. Seven were reviewed in clinic for deep phenotyping by the first author and the responsible clinician. For the others, comprehensive data were collected from the responsible clinicians. Clinical information for each patient is detailed in the supplementary information.

Eight recruits were female and 6 were male. Eleven had a *de novo* variant and 1 had a maternally inherited variant. Two patients were monozygotic twins. Two patients were

brothers but the *IQSEC2* variant was not identified in their mother's blood sample. Ten recruits were reported as having a pathogenic- or likely pathogenic- variant and two had a variant reported as being of uncertain clinical significance, but with a phenotype consistent with that of the *IQSEC2*-related disorder and suspected by the responsible clinician to be causative of the disorder.

Amongst this cohort, there were no consanguineous parental unions. There was an increase in nuchal translucency in one case. Two recruits were born at 36 weeks' gestation and the remaining singletons were born at 37-42 weeks' gestation. All singleton recruits had birth weights between 9th and 99th centiles. Their post natal heights were variable, between 0.4th and 75th centiles.

14/14 recruits (100%) had developmental delay and intellectual disability, which was severe or profound in all except one case (patient 7, who had moderate intellectual disability). The other features were: epilepsy, in 11/14 (78.6%), ataxic gait, in 7/9 who were able to walk (77.8%), stereotypies, in 10/14 (71.4%), drooling and post natal microcephaly (defined as third centile or below), each in 9/14 (64.2%), constipation and tone abnormality, each in 8/14 (57.1%), developmental regression in at least 7/14 (50%), hypotonia and feeding difficulties, each in 7/14 (50%), variable structural brain abnormalities, in 6 of 13 who underwent brain MRI (46.2%), gastro-eosophageal reflux, in 6/14 (42.9%), brachycephaly and autism, each in 5/14 (35.7%), scoliosis, paroxysms of laughter and eczema, each in 4/14 (28.6%), and plagiocephaly, cortical blindness and gastrostomy requirement, each in 2/14 (14.3%).

Epilepsy was generally diagnosed between the ages of 1 and 3 years in this cohort. The patients showed a variety of different seizure types, although generalised tonic-clonic was the most common. Control of the seizures ranged from completely controlled to intractable. Two of the 3 patients who did not have seizures had a history of vacant episodes. Stereotypies included teeth gnashing, bruxism, hand flapping, hand shaking,

hand wringing, hand 'wiping', head banging, head shaking and rocking. Most patients who had stereotypies displayed more than one type.

The most commonly requested genomic investigations prior to recruitment to the DDD study were for Angelman syndrome and Rett syndrome, each in 5/14 (35.7%).

X inactivation studies on patients 6 and 7 and the mother of patient 5 showed no evidence of skewed X inactivation.

Features of patients in this cohort are summarised in table 1.

[insert *IQSEC2* table patients 1-7 followed by *IQSEC* table patients 8-14]

Photographs of the patients are shown in figure 1.

[insert figure 1]

Discussion

Delineating the phenotype

Developmental delay and intellectual disability were the most prevalent features of this cohort, affecting all recruits. Also highly prevalent in this cohort were: stereotypies, epilepsy, microcephaly, developmental regression, hypotonia and autism- in agreement with previous reports[1],[4],[9],[10],[11].

Drooling and paroxysms of laughter, present in 64.2% and 28.6%, respectively, in this cohort, have not been previously reported in association with *IQSEC2* variants and appear likely to represent important features of the condition. Drooling in this cohort may be due to oral motor dysfunction and hypotonia.

There has only been one report each in the published literature of ataxia[12] and brachycephaly[9], present in 77.8% and 28.6%, respectively in this cohort. None of the

patients with ataxia had cerebellar abnormalities on the MRI that could account for their ataxic gait.

Structural brain abnormalities, present in 46.2% were common in recruits to this study, in agreement with previous reports[4],[9]. There did not appear to be any definite correlation between the presence of a structural brain abnormality and the extent of developmental delay, sex of the patient, type of variant present or whether or not microcephaly was present. Scoliosis and early feeding difficulties, present in 28.6% and 50%, respectively were also prevalent in this cohort. There have been previous reports of scoliosis in three individuals with *IQSEC2* variants[4],[13] and feeding difficulties have also been previously reported in three patients with *IQSEC2* variants[13],[1],[10], including one who required a gastrostomy[13]. The findings, taken together with previous reports suggest that these features are likely to form part of the *IQSEC2*-related disorder. The requirement for gastrostomy feeding in two patients in this cohort was likely due to a combination of developmental delay / intellectual disability, hypotonia and oral motor dysfunction. These patients did not have early feeding difficulties, implying that early feeding difficulties are not an indicator of the likelihood of requiring gastrostomy feeding later in childhood in the *IQSEC2*-related disorder.

Plagiocephaly, cortical blindness and strabismus were also found in 16.7% of this cohort and may be associated with the disorder. Amongst individuals with *IQSEC2* variants, cortical blindness has not been previously reported; plagiocephaly has been previously reported in two patients[9] and strabismus has previously been reported in 16 individuals[4],[11],[13],[1],[9],[10],[14],[15].

Constipation, gastro-oesophageal reflux and eczema, whilst common in all children appeared to be over-represented in recruits to this study. Amongst individuals with *IQSEC2* variants, gastro-oesophageal reflux has only been reported in one case[15] and constipation and eczema have not been reported but data from this study support an association.

Many patients did not have their occipital frontal circumference (OFC) at birth documented. However, patients 2,10,12 and 14, who had serial OFC measurements in their early childhood, demonstrate a 'crossing of the centiles' towards 0.4th centile and provide evidence that the microcephaly seen in the *IQSEC2*-related disorder is, at least in some cases, secondary microcephaly. This is consistent with a previous report[13]. Secondary microcephaly in individuals with *IQSEC2* variants implies a brain growth deceleration phase following birth and this may be extremely marked, as demonstrated by the serial measurements of patients 12 and 14.

Regression of development is frequently observed in children with seizures. The observations that patient 3 displayed regression prior to the onset of seizures and patient 5 lost skills despite not having seizures provides evidence that regression of development is a distinct feature of the *IQSEC2*-related disorder, rather than merely being the consequence of epilepsy. It is likely to be difficult to assess for developmental regression in patients who have not achieved any speech or significant motor skills beyond a social smile.

Posterior urethral valves (patient 5) and precocious puberty (patient 11) have not been previously reported in the context of an *IQSEC2* variant and it remains to be seen whether or not they form part of the condition.

In previous reports, dysmorphic facial features were present in some individuals with *IQSEC2* variants and not others. The same was true of this cohort. When the clinical photographs of the patients were reviewed together, common facial features were observed. Patients 2, 3, 6 and 12 were noted to have deep-set eyes. Patients 2, 3, 4, 5, 6, 9, 12 and 13 had full lips. Patients 2, 6, 12, 13 and 14 had a frontal upsweep of hair. Whilst there is not a very obvious gestalt for the *IQSEC2* syndrome, the facial features and hair patterning, together with the presenting clinical problems may provide clues to the clinician as to the diagnosis in the child with an undiagnosed *IQSEC2* variant.

Males in this cohort had more significant motor delay, intellectual disability and visual impairment than the females. It is thought that males with loss of function variants always present with severe intellectual disability, epilepsy and are non-verbal[16] and this was supported by our findings. Interestingly, females in this cohort were more likely than the males to receive a diagnosis of autism; this is likely because the extent of the disability in males might preclude the possibility of assessment for autism. Male patient 2 and female patient 4 have the same variant, however patient 2 has much more severe developmental delay. (Patient 4 first sat independently at 12 months and walked at 23 months and was able, at her best, to speak many words and use cutlery, whereas patient 2 is yet to sit up unaided at the age of 6 years and is non-verbal). Also the female twins (patients 6 and 7) harboured the same variant as the brothers (patients 13 and 14), however the brothers had more significant physical and intellectual disability than the girls. Comparison of these patients provides further evidence for a more severe male phenotype.

Tran Mau-Them *et al*. hypothesised that patients with truncating *IQSEC2* variants have a more severe phenotype when compared to those with missense variants[11], suggesting that the phenotype arises due to loss of function. It has been suggested that abolition (rather than a reduced level) of enzymatic activity of IQSEC2 typically leads to a more severe phenotype, including epileptic encephalopathy in both sexes[11]. However, this is not necessarily the case in this cohort, with patient 3 avoiding epileptic encephalopathy, despite having absent IQSEC2 protein; and patient 2 having a very severe phenotype, despite his variant being a missense (albeit in the critical sec7 domain). These observations support the notion that it is the sex of the patient and/or the protein domain disrupted by the variant that is more predictive of the severity of the phenotype.

Patient 5, who has severe intellectual disability but no seizures, has a variant of uncertain clinical significance (VUS) in a region of *IQSEC2* not known to be of functional importance. Previously, missense variants have been reported in males with severe

intellectual disability but not seizures[11],[17]. The fact that the mother of patient 5 (with normal intelligence) had a normal X inactivation pattern does not exclude pathogenicity of his variant because inheritance of *IQSEC2* missense variant from a non-epileptic, normal intelligence female has been reported[13], as has inheritance from females with only mild learning difficulties[18],[19],[16]. The variants of patients 1 and 5 have not been previously reported and there does remain some uncertainty as to their pathogenicity. However, many *IQSEC2* missense variants in regions not known to be functional have been described in patients with intellectual disability, with or without epilepsy[16]. If these variants are shown to be causative of their disorder, then this implies that there exist other, as yet unrecognised important functional domains of the IQSEC2 protein.

Explaining the female *IQSEC2* phenotype

It has been challenging to find an explanation for the *IQSEC2* phenotype in females[4],[16], given that females can have a severe phenotype, despite having a 'back up' X chromosome. It has been suggested that, in females, haploinsufficiency is sufficient to produce the full phenotype or alternatively, these variants produce a dominant negative effect. These mechanisms imply X-linked dominance, so it is difficult to explain then, how some females with *IQSEC2* missense variants have a normal- or near normal phenotype, which would imply X-linked recessive inheritance. This raises the possibility that *IQSEC2* variants can show X-linked dominant or X-linked recessive inheritance and this may depend on the type of variant (missense versus nonsense or frameshift).

IQSEC2 is widely quoted in the literature to 'escape' X inactivation (i.e. is expressed from both copies of the X chromosome in females), based on the findings of Tsuchiya *et al.*[20] and Cotton *et al.*[21], although *IQSEC2* is not specifically mentioned in these papers. This view seemed to be in agreement with the finding that IQSEC2 is expressed in very similar levels in human female and male brain[22]. However, other work had shown that *IQSEC2* is X-inactivated[23] and X-inactivation studies cast doubt on the escape theory. Of the 4 patients in the literature where X inactivation studies have been performed on samples of

females with *IQSEC2* variants, one had unfavourable skewing of 100:0 in blood[1], two others had severely unfavourably skewed X inactivation (100:0 and 97:3)[4] and a fourth had normal (66:34) X inactivation[4]. That skewed X inactivation is a principal moderator of phenotype in the *IQSEC2*-related disorder was not supported by the results of X inactivation studies performed on patients 6 and 7 and of the mother of patient 5 in this study. However, this is a small sample size and these results may not reflect the pattern of X inactivation in other tissues. It may be that *IQSEC2* may be variably inactivated[16], as is known to be the case for many other genes.

The IQSEC2-related disorder mimics other syndromes

There is a well-documented similarity in phenotype of individuals with *IQSEC2* variants and Rett syndrome[11],[4],[24]. In agreement with this, 5 (35.7%) of this cohort had had *MECP2* analysis, prior to the identification of the *IQSEC2* variant.

Other genes noted to have a phenotypic overlap with the *IQSEC2*-related disorder include *FOXG1*, *CDKL5* and *MEF2C*[24]. Consistent with this, was the fact that one sample from this cohort had *MEF2C* testing prior to the identification of the patient's *IQSEC2* variant, one other underwent analysis of *FOXG1* analysis and three others underwent *CDKL5* analysis. *CDKL5* and *IQSEC2* are expressed in the same areas of the brain, suggesting a functional link between the two proteins[1].

Five (35.7%) patients in this cohort had been previously tested for Angelman syndrome. A phenotypic overlap of those with *IQSEC2* variants and Angelman syndrome has not been previously discussed in the medical literature, although Morleo *et al*. noted that there was partial overlap with the Angelman phenotype in their patient[1] and some patients with *IQSEC2* variants were noted to have had prior genomic analysis for Angelman syndrome[13],[11],[24]. Since 5-10% of patients with suspected Angelman syndrome do not have the classical associated chromosome 15q11 methylation changes or *UBE3A* variant[14], *IQSEC2* analysis in patients with an Angelman-like phenotype may represent an opportunity for diagnosis of the *IQSEC2*-related disorder. This may be

more relevant for female patients, since most patients with Angelman syndrome learn to walk and males with *IQSEC2* variants often do not, although two patients tested for Angelman syndrome in this cohort were male. In males, the overlap is most likely to be greater at a younger age, following which the phenotypes of Angelman syndrome and the *IQSEC2*-related disorder diverge.

IQSEC2 is included on some -but not all- developmental delay gene panels or subpanels available in the UK. Most epilepsy panels do not include *IQSEC2*[25]. The addition of *IQSEC2* to these panels is likely to reveal more cases of the *IQSEC2*-related disorder.

Gonadal mosaicism

The finding of the same *IQSEC2* variant in samples from patients 13 and 14 but not their mother's sample implies that their mother displays gonadal mosaicism for the *IQSEC2* variant. There has been one previous report of gonadal mosaicism in a family with the *IQSEC2*-related disorder[26]. We therefore recommend that parents of a child with the *IQSEC2*-related disorder should be counselled for the potential for gonadal mosaicism and the potential recurrence risk (which currently cannot be quantified) in subsequent pregnancies.

Twin phenotype discordance

The phenomenon of twin discordance for features of the *IQSEC2*-related disorder has not been reported previously. Patient 6 has profound intellectual disability with epilepsy and aggressive / self-injurious behaviour, whereas her monozygotic twin sister, patient 7, has a much milder phenotype, with the ability to attend a mainstream school until the age of 10 years. Indeed, patient 7 had never been referred to a community paediatrics or genetics clinic prior to the identification of the *IQSEC2* variant in her twin sister. The twins were also discordant for other features distinct from the *IQSEC2* phenotype, namely birth weight, face shape, patency of the ductus arteriosus and weight.

Monozygotic twins are not truly identical and X inactivation and imprinting are known to be discordant in some monozygotic twin pairs[27] (non-concordance for Beckwith–Wiedemann syndrome amongst monozygotic twins is a well-known example[27]). Hogenson[28] argues that epigenetic alterations are the principal mechanism responsible for twin discordance. Machin[27] provides many examples from the medical literature of monozygotic twins discordant for genetic conditions, most of which are not explained by a post zygotic variant in the affected twin. The value of studying discordant monozygotic twin sets to investigate the effects of epigenetic alterations has been realised[29] and epigenetic findings from disease-discordant monozygotic twin studies so far have identified DNA methylation changes in multiple genes across a wide range of conditions. Epigenetic mechanisms, examples of which include DNA methylation, histone acetylation, chromatin remodelling and microRNA expression, induce or suppress gene expression, potentially altering the phenotype. Epigenetic alterations are often caused by an environmental influence and are dynamic throughout life. Patient 7's developmental milestones were mostly normal, implying that any epigenetic change responsible for her attenuated phenotype occurred in very early life or *in utero*.

Twin non-concordance can, in some cases be explained by the non-equal allocation to each twin of stem cells after formation of the blastomere[27], which may have important implications for the cascade of developmental events during embryogenesis[30]. Weksberg *et al.*[30] hypothesize that post zygotic events may lead to the formation of two or more cell clones in the inner cell mass of the embryo and that this actually precipitates the twinning event. Other potential explanations for twin discordance in this cohort include post zygotic or somatic single nucleotide polymorphisms, that either directly –and coincidentally- affect IQSEC2 or proteins with which it interacts, or themselves cause the alteration of epigenetic marks[29].

The observation that DNA methylation profiles are less alike within pairs of monochorionic, monozygotic twins compared to pairs of dichorionic, monozygotic twins

suggests that sharing a placenta may cause unequal conditions in fetal life and therefore more discordant epigenetic profiles[29]. It remains unknown whether or not the putative twin-to-twin transfusion played a role in differing epigenetic profiles or contributed to the disparity in phenotype.

Future Therapies

Recently, the goal of curing genetic diseases has edged closer to becoming a reality, with the discovery and utilisation of the CRISPR (clustered regularly interspaced short palindromic repeats) Cas9 system to repair missense variants and cure genetic disease[31]. The finding that the shorter *IQSEC2* isoform, shows continued and increasing expression in post natal life[32] offers hope that amelioration of the severity of the *IQSEC2* phenotype may be possible by means of repairing of missense variants in *IQSEC2* or using drug therapy to inhibit epigenetic marks. The latter would depend on the identification of epigenetic marks that are capable of moderating the phenotype in this disorder. Secondary microcephaly in individuals with *IQSEC2* variants implies a brain growth deceleration phase following birth (in at least some cases), meaning that there may be a window of opportunity early in life in which to treat these individuals with any such targeted therapy.

Conclusion

The data from this study provide evidence for several new features of the *IQSEC2*-related disorder and support the notion of an emerging *IQSEC2* syndrome. We echo a previous recommendation by Helm and colleagues[13] to consider requesting *IQSEC2* analysis in all patients with severe intellectual disability and seizures or a phenotype resembling Rett syndrome. In addition, we suggest that firstly, the possibility of the presence of an *IQSEC2* variant is also considered in patients with an Angelman- / Pitt-Hopkins-like phenotype and secondly, that *IQSEC2* should be added to epilepsy- and developmental delay / intellectual disability panels. As whole exome- and whole genome

sequencing become more widely available, it is likely that an increasing number of *IQSEC2* variants will be identified.

The observation of apparent gonadal mosaicism in this cohort has implications for genetic counselling of parents with an affected child.

The observation of twin discordance in phenotype in this study offers hope for the possible development of a therapy to mitigate the severity of the *IQSEC2* phenotype.

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Figure 1: Photographs of patients in this study. Patients 6 and 7 are monozygotic twins. Patients 13 and 14 are brothers. Patients 1 and 5 are the patients with the missense variants. Patients 2, 3, 4, 6, 10, 12 and 13 were noted to have full lips. Patients 2, 3, 10 and 12 had deeply-set eyes. Patients 2, 6, 12, 13 and 14 had frontal upsweeps of hair.

Table 1: summary of genotype, phenotype and previous genomic analysis for each patient.

Key: PVL = periventricular leucomalacia.

Patient	1	2	3
variant	c.3412G>C p.(Gly1138Arg)	c.2507C>T p.(Ala836Val)	c.1591C>T p.(Arg531*)
type	missense	missense	nonsense
protein	*in silico* inconclusive	sec7 domain altered	absent
class	3	4	5
relevant family history	nil	nil	nil
inheritance	*de novo*	*de novo*	*de novo*
sex	female	male	female
birth OFC	unknown	unknown	unknown
post natal OFC centile	51st (9yr)	22nd (13mo) 3rd (6 yr)	1st (12yr)
Tone	normal	upper limb spasticity	hypotonia
early feeding difficulty	no	yes	yes
seizures	photosensive tonic-clonic	tonic-clonic	tonic-clonic absence partial status
autism	yes	no	yes
social smile	10 wk	unknown	unknown
rolled / sat / crawled	sat 10 mo	N/A	sat 9 mo
walked	1 yr	N/A	15 mo
first words	15 mo	N/A	11 mo
regression	yes	no	yes
reflux	no	no	yes
constipation	yes	yes	yes
drooling	yes	yes	no
stereotypies	no	yes	yes
dysmorphic features	none	yes	no
scoliosis	yes	yes	yes, severe
plagiocephaly	no	yes	no
brachycephaly	no	yes	no
other skeletal	clinodactyly	camptodactyly	none
gait	ataxic	N/A	ataxic
demeanour		happy	bouts of laughter
aggression	no	no	no
sleep difficulty	yes	no	no
other	ADHD eczema	cortical blindness diastasis recti	eczema

	anxiety	eczema	
genetic tests	*PCDH19*	Angelman	Angelman *MECP2* *FMR1* *CDKL5* telomere analysis epilepsy and severe delay panel
MRI brain	normal	mild brain atrophy	normal

4	5	6
c.2507C>T	c.2117A>G	c.4419_4420insC
p.(Ala836Val)	p.(Asn706Ser)	p.(Ser1474Gln fs*)
missense	missense	frameshift
deleterious/disease-causing	*in silico* inconclusive	elongated
4	3	4
nil	nil	twin sister affected
de novo	maternally	*de novo*
female	male	female
1st	unknown	unknown
1st (6 yr)	9th (10 wk)	1st (10yr)
	0.4th (13 yr)	
normal	hypotonia	hypotonia
no	yes	no
tonic-clonic	no	absence
		complex
no	no	yes
6 wk	unknown	expected time
sat 1 yr	sat 13 mo, crawled 23 mo	sat 2 yr
23 mo	6 yr	4.5 yr
18 mo	6 yr	7 yr
yes, marked	yes	yes
no	yes	yes
no	no	no
no	yes	yes
yes	yes	yes
yes	yes	no
yes	no	no
no	no	no
no	yes	no
none	none	none
ataxic when younger	ataxic	ataxic
	happy, friendly	bouts of laughter
no	no	yes + self injurious
no	no	yes
breathing	vacant episodes	
abnormalities	posterior	

	urethral kidney valves	
MEF2C	Angelman	Angelman
PWS	*SLC9A6*	*MECP2*
TCF4		
normal	normal	hypomyelination of white matter

7	
c.4419_4420insC	
p.(Ser1474Gln fs*)	
frameshift	
elongated	
4	
twin sister affected	
de novo	
female	
unknown	
<0.4th (10yr)	
normal	
no	
no	
yes	
3 mo	
sat 7 mo	
15 mo	
11.5 mo	
no	
no	
no	
no	
no	
no	
no	
no	
no	
none	
ataxic	
shy	
no	
yes	
N/A	

none

N/A

Patient	8	9
variant	c.2911C>T p.(Arg971Ter)	c.325delC insGC p.(Gln1084Ala fs Ter22)
type	nonsense	frameshift
protein	truncated	truncated
class	5	4
relevant family history	brother has epilepsy, autism	nil
inheritance	*de novo*	*de novo*
sex	female	male
birth OFC	unknown	unknown
post natal OFC centile	2nd (17 mo)	40th (17yr)
Tone	normal	normal
early feeding difficulty	no	yes
seizures	yes	tonic clonic
autism	no	no
social smile	8 wk	unknown
rolled / sat / crawled	sat 2-2.5 yr	N/A
walked		N/A
first words		N/A
regression	no	no
reflux	no	no
constipation	yes	yes
drooling	no	yes
stereotypies	yes	yes
dysmorphic features	yes	no
scoliosis	no	no
plagiocephaly	yes	no
brachycephaly	yes	no
other skeletal	none	none
gait	normal	N/A
demeanour		
aggression	no	no
sleep difficulty	no	no
other		apnoeas paroxysmal dystonia gastrostomy optic atrophy

genetic tests	Angelman	*MECP2* *CDKL5*
MRI brain	hypoplastic frontal lobes	cerebral atrophy intracranial cystic lesion

10	11	12
c.1556_1599delACCT	c.2911C>T	c.847_848del insT
p.(Tyr519Trp fsTer87)	p.(Arg971Ter)	p.(Gly283Ser fs*23)
frameshift	nonsense	frameshift
truncated	truncated	truncated
4	4	4
nil	nil	nil
do novo	*de novo*	*de novo*
female	female	male
unknown	unknown	unknown
38th (19 mo)	94th (14.5 yr)	90th (21 mo)
1st (5.5 yr)		7th (8yr)
hypotonia	normal	hypotonia
yes	no	no
no	yes	intractable tonic-clonic
no	yes	some features
6 wk	20 wk	5 wk
sat 18 mo	sat 1 yr	rolled 5-6 mo
22 mo	2-2.5 yr	N/A
2 yr	2.5-3 yr	N/A
no	possible	yes
no	no	yes, severe
no	no	yes
no	yes	yes
yes	yes	yes
yes	yes	no
no	no	no
no	no	no
no	no	yes
none	none	none
toe walking	ataxic	N/A
	happy, bouts of laughter	bouts of laughter
no	yes	no
no	no	no
squint	precocious puberty	cortical blindness
hypermetropia	anxiety	squint
vacant episodes	skin picking	eczema
		dysplasia of hips / knees
		gastrostomy

nil	none	none
normal	arachnoid cyst	white matter loss PVL / hypoxic injury cortical dysplasia

13	14
c.4419_4420 insC	c.4419_4420 insC
p.Ser1474Gln fsTer133	p.Ser1474Gln fsTer133
frameshift	frameshift
elongation	elongation
4	4
brother affected	brother affected
mat gonadal mosaicism	mat gonadal mosaicism
male	male
unknown	59th
4th (9years)	1st (22 mo)
hypotonia	hypotonia
yes	yes
generalised myoclonic	generalised myoclonic
no	no
unknown	8 wk
sat 13 mo	N/A
N/A	N/A
N/A	N/A
yes	stagnation
yes	yes
yes	yes
yes	yes
no	no
yes	yes
no	no
no	no
no	yes
none	talipes
N/A	N/A
content	content
no	no
yes	no
	amyblyopia

MECP2	*CDKL5*
CNTNAP2	*TCF4*
ARX	*ARX*
MPHOSH6	*FOXG1*
mitochondrial	*MECP2*
	FMR1
normal	normal