Heterozygous truncation mutations of the *SMC1A* gene cause a severe early onset epilepsy with cluster seizures in females: detailed phenotyping of 10 new cases

Joseph D Symonds[1,2], Shelagh Joss[3], Kay A Metcalfe[4,5], Suresh Somarathi[4], Jamie Cruden[6], Anita M Devlin[7], Alan Donaldson[8], Nataliya DiDonato[9], David Fitzpatrick[10], Frank J Kaiser[11], Anne K Lampe[12], Melissa M Lees[13], Ailsa McLellan[14], Tara Montgomery[15], Vivek Mundada[16], Lesley Nairn[17], Ajoy Sarkar[18], Jens Schallner[19], Jelena Pozojevic[11], Ilaria Parenti[11], Jeen Tan[20], Peter Turnpenny[21], William P Whitehouse[18,22], The DDD Study[23], Sameer M Zuberi[1,2 *]

1. The Paediatric Neurosciences Research Group, Royal Hospital for Children, Queen Elizabeth University Hospitals, Glasgow, UK
2. School of Medicine, University of Glasgow, Glasgow, UK
3. West of Scotland Clinical Genetics Service, Glasgow, UK
4. Manchester Centre for Genomic Medicine, Manchester Academic Health Sciences Centre Manchester, UK
5. Division of Evolution and Genomic sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK
6. Department of Paediatrics, Victoria Infirmary, Kirkcaldy, UK
7. Paediatric Neurology, Great North Children's Hospital, Newcastle Acute Hospitals NHS Trust, Newcastle-upon-Tyne, UK
8. University of Bristol, Bristol, UK
9. Institute for Clinical Genetics, TU Dresden, Dresden, Germany
10. MRC Human Genetics Unit, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK
11. Section for Functional Genetics, Institute for Human Genetics, University of Lübeck, Lübeck, Germany
12. South East Scotland Clinical Genetic Service, Edinburgh, UK
13. Clinical Genetics, Great Ormond Street Hospital, London, UK
14. Department of Paediatric Neurosciences, Royal Hospital for Sick Children, Edinburgh, UK
15. Institute of Genetic Medicine, Newcastle-upon-Tyne, UK
16. Paediatric Neurology Royal London Hospital, London, UK
17. Department of Paediatrics, Royal Alexandra Hospital, Paisley, UK
18. Nottingham University Hospitals NHS Trust, Nottingham, UK
19. Universitätsklinikum Carl Gustav Carus an der TU Dresden, Dresden, Germany
20. Paediatric Neurology, Royal Manchester Children's Hospital, Manchester, UK
21. Peninsula Genetics, Exeter, UK
22. School of Medicine, University of Nottingham, UK
23. The Deciphering Developmental Disorders study, Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK

***Correspondence:**
Professor Sameer M Zuberi, The Paediatric Neurosciences Research Group, Royal Hospital for Children, Queen Elizabeth University Hospitals, 1345 Govan Road, Glasgow, UK, tel: +44 (0) 141 451 6521, fax: +44 (0) 141 201 2215, email: sameer.zuberi@nhs.net

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Abstract

Objective: The phenotype of seizure clustering with febrile illnesses in infancy/early childhood is well-recognized. To date the only genetic epilepsy consistently associated with this phenotype is *PCDH19,* an X-linked disorder restricted to females, and males with mosaicism.

The *SMC1A* gene, which encodes a structural component of the cohesin complex is also located on the X chromosome. Missense variants and small in frame deletions of *SMC1A* cause approximately 5% of Cornelia de Lange Syndrome (CdLS).

Recently, protein truncating mutations in *SMC1A* have been reported in five females, all of whom have been affected by a drug-resistant epilepsy, and severe developmental impairment. Our objective was to further delineate the phenotype of *SMC1A* truncation.

Method: Female cases with *de novo* truncation mutations in *SMC1A* were identified from the Deciphering Developmental Disorders (DDD) study (n=8), from postmortem testing of an affected twin (n=1), and from clinical testing with an epilepsy gene panel (n=1). Detailed information on the phenotype in each case was obtained.

Results: 10 cases with heterozygous *de novo* mutations in the *SMC1A* gene are presented. All 10 mutations identified are predicted to result in premature truncation of the *SMC1A* protein. All cases are female, and none had a clinical diagnosis of CdLS. They presented with onset of epileptic seizures between <4 weeks and 28 months of age. In the majority of cases a marked preponderance for seizures to occur in clusters was noted. Seizure clusters were associated with developmental regression. In all cases moderate or severe developmental impairment was apparent.

Significance: Truncation mutations in *SMC1A* cause a severe epilepsy phenotype with cluster seizures in females. These mutations are likely to be non-viable in males.

Introduction

A number of X-linked epilepsies in which females are exclusively, or disproportionately, affected have been described. The most well-delineated is *PCDH19*-related epilepsy, a condition which typically presents with fever-sensitive focal seizures, often occurring in clusters, and in which developmental problems often become apparent after epilepsy-onset[1]. Other examples include *CDKL5*[2], *KIAA2022*[3], *HNRNPH2*[4], and *CASK*[5]. In contrast to *PCDH19*, in which (non-mosaic) male mutation carriers are usually asymptomatic, in *CDKL5*, *HNRNPH2, KIAA2022*, and *CASK*, males are presumed to be non-viable, though rare cases of more severely affected males have been described in *CDKL5*, *KIAA2022*, and *CASK*[2,3,5].

The *SMC1A* gene, located at Xp11.22, encodes one of four core subunits that make up the cohesin ring. The cohesin ring plays important roles in cell division, transcription regulation, and DNA repair[6]. Derangements of the cohesin ring are known to cause Cornelia de Lange syndrome (CdLS), a multisystem developmental disorder first described in 1849[7]. Affected individuals are typically microcephalic with striking pre- and postnatal growth restriction often associated with feeding difficulties and gastro-esophageal reflux[8]. They have a characteristic facial appearance with fine arched eyebrows, synophrys, long philtrum, thin upper vermilion and low set posteriorly rotated ears and variable presence of malformations (limb, cardiac, diaphragmatic, gastrointestinal and genitourinary). The reported prevalence of epilepsy ranges from 4 to 23%, with no particular seizure pattern described[9].

Approximately 65% of CdLS cases are caused by mutations in genes (*NIPBL*, *SMC1A, SMC3*, *RAD21*, and *HDAC8*) which encode structural components or regulators of the cohesin ring[10]. To date 60 cases of CdLS due to *SMC1A* mutation have been described, with a male:female ratio of 1:2[11]. All reported variants have been missense mutations or small in-frame deletions. This led to the previous hypothesis that mutations causing truncation of the *SMC1A* protein would be either asymptomatic or non-viable[11].

The absence of *SMC1A* protein truncating mutations in large databases comprised largely of asymptomatic individuals favored the hypothesis that they would be non-viable[12]. However, recently, *SMC1A* truncation mutations have been described in five cases, all female, none of whom had a clinical diagnosis of CdLS[13-15]. All five cases developed drug-resistant epilepsy and had severe developmental impairment. Age at presentation with first epileptic seizure ranged from <1 month to 17 months. 4/5 cases were reported to have a clustering pattern to the seizures.

Here we report the phenotypes of 10 new cases of *SMC1A* truncation related epilepsy, further delineating the phenotype. Our data help further define *SMC1A* truncation as a genetic epilepsy with distinct electro-clinical features, that can occur in cases without typical features of CdLS. We propose that, along with *PCDH19*, testing of the *SMC1A* gene should be considered in females with early childhood onset drug-resistant epilepsy, particularly where there is a clustering pattern to the seizures.

Methods

Ethical compliance:

The DDD study has UK Research Ethics Committee approval (10/H0305/83, granted by the Cambridge South REC, and GEN/284/12 granted by the Republic of Ireland REC).

Case Ascertainment:

8 of the 10 affected cases (cases 1-8) were recruited via UK NHS Regional Genetics Services to the Deciphering Developmental Disorders (DDD) project (http://www.ddduk.org/). Case 9 was tested postmortem following the genetic result found in her twin sister through DDD. Case 10 was followed in pediatric neurology and genetics due to a severe developmental and epileptic encephalopathy.

A structured proforma, including detailed phenotypic data, was completed by both the clinical geneticist and the pediatric neurologist involved in each case; this was used in conjunction with information in the DDD database.

Sequencing and analysis

For cases 1-8, trio-based exome sequencing was performed as part of the DDD study as previously described[16,17]. Target capture using Agilent SureSelect 55 MB Exome Plus was performed on saliva- or blood-derived genomic DNA from each affected case and their parents and sequenced on Illumina HiSeq. DeNovoGear21 was used to identify de novo sequence variants and Ensembl Variant Effect Predictor (VEP version 2.6, http://www.ensembl.org/info/docs/tools/vep/index.html) was used to predict the effect of each genomic variant. PolyPhen-2 analysis was carried out at http://genetics.bwh.harvard.edu/pph2/. SIFT analysis was carried out at http://sift.bii.a-star.edu.sg/www/SIFT_seq_submit2.html(parameters used: Database UniProt-SwissProt2010_09; Median conservation of sequences 3.00; Remove sequences more than 90% identical). Genbank accession for *SMC1A* is NM_006306.

Case 9 was post mortem sequenced locally for the variant found in her sister following her sister's result through DDD. Case 10 was tested using a custom gene panel of >100 candidate genes or genes associated with the epileptic encephalopathy, microcephaly and genetic syndromes with seizures.

Results

Clinical findings

We obtained detailed phenotypic information on nine cases with *SMC1A* truncation. A further case who was more severely affected than all of the others (case 9), was the older sister of case 8. Case 9 died at

aged 11 months. We were unable to obtain details of her epilepsy other than that she began having seizures in the neonatal period. She had semilobar holoprosencephaly, partial anomalous pulmonary venous drainage, and dysmorphic facial features. Her sister, with the same mutation, had no structural organ anomalies, no dysmorphic features, and a normal MRI brain scan. All 10 cases identified were female, and they all presented with a drug-resistant epilepsy, though two cases eventually became seizure-free (case 2 from aged five years, and case 4 from aged seven years). Prior to, and after, their genetic diagnosis, none were considered as having Cornelia de Lange syndrome (see Supplementary Material for further clinical details of each case).

Development

Apart from the two cases who began having seizures in the neonatal period, concerns about developmental delay had clearly arisen prior to the onset of epileptic seizures. All 10 cases have moderate, severe, or profound developmental impairment, with all domains of development affected. None of the cases had any expressive language. One case (case 6) did develop babbling between one and two years of age before losing this ability following a 45-minute episode of tonic-clonic status epilepticus at the age of three years. None of the other cases were felt to have had any regression in their development associated with either epilepsy onset, or any period of poor seizure control. Four of the 10 cases have achieved independent mobility, albeit with significant delay. Three of the 10 cases have cerebral visual impairment.

Seizures

Age of onset of first seizure ranged from the neonatal period to 28 months of age (median 4.5 months). The first epileptic seizure was an afebrile generalized motor seizure (generalized tonic-clonic, or generalized clonic) in five cases, a focal seizure in two cases, a generalized tonic seizure in one case, and

generalized tonic-clonic febrile convulsion in one case. Seven cases demonstrated both focal and generalized seizure types, whilst two cases had generalized seizures only. Seven cases demonstrated a clear predisposition for seizures to occur in clusters. For example, case 6 currently has clusters of between three and 10 focal-onset seizures, occurring over a period of 24 hours, every 10-21 days, with complete seizure-freedom between clusters. Case 8 has clusters of 6-7 generalized tonic-clonic seizures occurring two to three times per month. Two cases have had episodes of convulsive status epilepticus, and one case has had episodes of non-convulsive status epilepticus.

Electroencephalogram (EEG)

All nine cases for whom we have seen EEGs or EEG reports have demonstrated focal or multifocal abnormalities. Typically, the interictal EEGs show independent multifocal spike and sharp-wave complexes seen in both awake and sleep recordings (see figures 6 and 8), as well as subclinical runs of generalized spike-wave abnormalities, lasting several seconds (Figure 1A). Ictal EEGs show generalized slowing of background (Figure 1C), followed by focal spike discharge widely distributed over one hemisphere (Figure 1E). In other EEGs in the same case the spike discharge may be over the other hemisphere.

Epilepsy treatment

From this small case series, no single anti-epileptic medication has emerged as clearly more efficacious than others. In fact, though six of the referrers reported one or more anti-epileptic medication to be particularly efficacious in their case, in all six cases this was a different drug. One case became seizure-free shortly after introduction of Phenobarbitone, one case became seizure-free shortly after introduction of Gabapentin, and one case achieved seizure-freedom for one year after Levetiracetam was started. In all cases multiple medications were tried (range 4-8). Three have tried the ketogenic diet,

two of whom were felt to benefit significantly in terms of seizure-control, and one of whom remains on it at present (after 4.5 years). Two have tried vagus nerve stimulation, both without perceived benefit.

Growth

Short stature and progressive microcephaly were seen in the majority of cases. Overall, height and head circumference are proportionally small. Most recent height measurement was >2 Z-scores in 7/9 cases below the mean on most recent height measurement, and mean most recent height was -3.0 Z-scores (SD 1.5). None of the cases had microcephaly at birth, though mean birth occipito-frontal circumference (OFC) was -1.5 Z-scores below the mean. Most recent OFC was >2 Z scores below the mean in 8/9 cases, with a mean most recent OFC of -3.0 Z-scores (SD 1.6). The cases also demonstrated a deceleration in weight gain. Only 1/10 cases had a low birth weight (>2 Z-scores below the mean), though mean birth weight for the 10 cases was -1.29 Z-scores (SD 0.67). However, in 5/9 cases the most recent weight was >2 Z-scores below then mean, and the mean most recent weight for the group was -2.2 Z-scores (SD 1.4).

Dysmorphism and associated anomalies

Two cases have no dysmorphic features and no congenital anomalies, and another one has dysmorphic features but no congenital anomalies. Four cases have congenital cardiac anomalies: two atrial septal defect *plus* ventricular septal defect, one atrial septal defect only, and one partial anomalous pulmonary venous drainage. Two cases have had cleft palate, and two have bifid thoracic vertebrae. From photographs the primary authors have seen, there appears to be a characteristic facial appearance, consisting of a flattened mid-face, a short, upturned nose, and a shallow philtrum (Figure 2).

Genetic findings

The phenotypic findings of the 10 cases from our study, along with those of the previously reported 5 cases are summarized in Table 1. The genetic findings of the 10 new cases are summarized in Table 2. In all 10 cases the mutation had arisen *de novo* and was predicted to lead to premature truncation of the *SMC1A* protein.

Discussion

Analysis of the 10 cases reported here, and the previously published five cases, allows delineation of a distinct epilepsy phenotype associated with *SMC1A* truncation mutations. Eight of our 10 cases were recruited from a large cohort who had been referred for genetic investigation of a developmental disorder (the DDD study), not specifically for epilepsy[17]. Despite the broad entry criteria for the DDD study, all the *SMC1A* truncation cases identified from this cohort have been found to have a severe drug-resistant epilepsy, suggesting that the correlation between *SMC1A* truncation and the epilepsy phenotype is strong. All five previously reported cases in the literature also had drug-resistant epilepsy[13-15]. Additionally, Hansen et al. reported a female with a *de novo* splice-site mutation in *SMC1A* who had infantile-onset epilepsy characterized by convulsive seizures "occurring in impressive clusters lasting 24-48 hours[18]." In their discussion Hansen et al. commented that although the diagnosis of Cornelia de Lange syndrome was not considered initially, "retrospectively the girl's symptoms and facial gestalt [were] compatible with mild Cornelia de Lange syndrome (CdLS)." We argue that *SMC1A* truncation does not represent a mild form for CdLS since these females are affected by a more severe developmental disorder than is typical for CdLS caused by *SMC1A* variants.

The presence of seizure clustering is a strong theme that has emerged in our cases. 7/10 cases were noted to have seizure-clustering. Seizure clustering was also noted in 4/5 of the previously reported

cases. The only other genetic epilepsy for which seizure clustering has emerged as a prominent feature is *PCDH19*-related epilepsy. As with *SMC1A*, *PCDH19* related epilepsy is an X-linked disorder, affecting mostly females. The *PCDH19* phenotype has now been well-defined, and can be summarized as follows: Normal development before onset of seizures; seizure onset between 3 months and 3 years (median 11 months)[19]; clusters of convulsive seizures which are markedly fever-sensitive; progression to multiple seizure types including focal seizures and absences[1]. Developmental stagnation or regression may be noted after epilepsy onset, though up to 40% of *PCDH19* cases continue to have normal development[19,20]. Dysmorphism and malformation are not commonly associated with *PCDH19*[21].

Epilepsy onset and seizure clustering is remarkably similar in these *SMC1A* cases to that seen in *PCDH19*-related epilepsy. Seizure onset was from <4 weeks to 28 months (median 5 months). Though bilateral motor seizures predominated, 9/15 had multiple seizure types. Despite certain similarities, a number of features distinguish *SMC1A* truncation-related epilepsy from *PCDH19*. From our 10 cases and the 5 previously reported cases, 12/15 were noted to have dysmorphic features and 6/15 had malformations (cardiac, vertebral, palatal). These cases are also notable for short stature, a marked progressive microcephaly, and for the presence of a moderate to severe developmental impairment which clearly preceded seizures in those cases with later-onset epilepsy. In terms of their epileptic seizures, these *SMC1A* truncation cases do not appear to demonstrate the same degree of fever-sensitivity as is seen in *PCDH19*. Furthermore, myoclonic seizures, hemiclonic seizures, and tonic seizures were reported in multiple cases in this series, but these seizure types are not typically seen in *PCDH19*-related epilepsy[20].

There are phenotypic similarities between the cases reported here and CdLS, including short stature, microcephaly and developmental delay, as well as dysmorphic features and congenital anomalies in some. Four of our cases had structural cardiac anomalies, including atrial septal defect (ASD) and ventricular septal defect (VSD). A wide variety of structural cardiac defects are seen in CdLS, including

ASD and VSD. Pulmonary stenosis, not seen in any of our cases, is the most frequently reported cardiovascular anomaly in CdLS[22,23]. Bifid vertebrae, seen in two of our cases, are not a frequently reported feature of CdLS[11].

What appears to distinguish *SMC1A* truncation carriers most from the typical CdLS phenotype is the absence of the characteristic facial features, and the severity of the epilepsy and developmental disorder. Though not a core feature of the syndrome, epilepsy is seen more frequently in CdLS than in the general population, with the estimated prevalence in various reports ranging from 4% to 23%[9]. Though epilepsy does not appear to be more frequently seen in *SMC1A*-related CdLS compared to CdLS due to mutations in other cohesin complex genes[10], it is notable that the majority of, though not all, male *SMC1A* cases reported have epilepsy as a feature[24-27].

Whether *SMC1A* truncation should be considered a separate entity to CdLS, or as a subtype of CdLS, is a matter for debate. Importantly, we have shown that *SMC1A* truncation can cause a severe developmental disorder and drug-resistant epilepsy in cases where CdLS was not otherwise considered. This may have implications for practice when clinicians are considering, and interpreting, genetic testing.

The underlying pathophysiology of CdLS caused by *SMC1A* mutations is not fully understood. *SMC1A* is a structural component of the cohesin ring, which binds to chromatin and plays an important role in the transcriptional regulation of a large number of other genes[28]. There are 60 published cases of *SMC1A*-related CdLS (35 female and 25 male), and all reported variants are either missense variants, or deletions that preserve the reading frame of the protein[11,24-27, 29-33]. In those cases in which there is familial inheritance of an *SMC1A* mutation, males demonstrate a more severe phenotype than females[24]. Functional studies using human lymphoblastoid cell lines of CdLS patients and healthy controls have demonstrated that in CdLS mutant *SMC1A* is almost fully transcribed and incorporated into the cohesin complex[29]. Since mutant *SMC1A* would be present in all male cells, whereas in females

it would only be present in those cells in which the mutated *SMC1A* allele is not inactivated, this could explain why male *SMC1A* CdLS patients appear to be more severely affected than females within the same family. However, *SMC1A* appears to escape inactivation in some, but not all, females[34], leading to a hypothesis that mutant *SMC1A* may exert a dominant negative effect in female CdLS cases[35].

The absence of any reported males with *SMC1A* truncation mutations implies that complete *SMC1A* deficiency is incompatible with viability. The mechanism of pathogenesis in females with *SMC1A* truncation can only be speculated. Lebrun et al.[15] demonstrated that in their female case of *SMC1A* loss-of-function there was a reduced level of *SMC1A* transcript. However, if *SMC1A* does escape X-inactivation then haploinsufficiency is unlikely to be the causative mechanism since female haploinsufficient cells would have the equivalent of a normal male complement of *SMC1A*[36]. It is possible that truncated *SMC1A* is transcribed and translated, escapes nonsense-mediated decay, and exerts a dominant negative effect. To gain a greater understanding of the mechanism would require full investigation of human *SMC1A* inactivation (including specifically in relevant tissues) and the extent to which truncated transcripts undergo nonsense mediated decay. Another valuable line of inquiry would be further definition of the role of *SMC1A* as a transcriptional regulator, whether such transcriptional regulation follows a cyclical pattern, and how this may relate to the clustering pattern of seizures seen.

Finally, it is interesting to note that despite a recent proliferation of large exome sequencing studies investigating children with early-onset epilepsy, *SMC1A* has not emerged as an important gene until now. One reason for this may be that recruitment to previous studies has focused on defined electro-clinical syndromes such as Ohtahara Syndrome, Infantile Spasms, Dravet Syndrome, Lennox Gastaut Syndrome, and Epilepsy with Myoclonic Atonic Seizures[37-41]. This series demonstrates that through the investigation of a broader group of children with developmental disorders, genetic syndromes with well-defined phenotypes can emerge.

Summary

SMC1A truncation mutations are only seen in females, and cause a condition in which the typical features of Cornelia de Lange syndrome are often absent. These females are all affected by a moderate-to-severe developmental impairment and a drug-resistant epilepsy which characteristically demonstrates a clustering pattern. Further investigation into how *SMC1A* truncation leads to this phenotype is required. It is likely that disrupted transcriptional regulation of other genes plays an important role.

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Disclosure

None of the authors have any disclosures to make in relation to this manuscript.

Ethics statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Keywords

SMC1A, epilepsy, cluster, X-linked, females, intellectual disability

Key points

- Truncation mutations in *SMC1A* have only been reported in females
- This series takes the total number of published cases to 15
- Early-childhood onset epilepsy and moderate-severe intellectual disability is seen in all cases
- Focal and generalized seizures are seen
- Seizures frequently occur in clusters, without a clear precipitant to the clusters
- Cases often lack the typical features of Cornelia de Lange Syndrome, which is caused by missense mutations in *SMC1A*

Figures and tables

- Table 1 - Summary of the phenotypic features from our 10 cases and the previously reported 5 cases
- Table 2 - Summary of genetic findings from our 10 cases
- Figure 1- EEGs from 3 cases
    - (A) Inter ictal sleep EEG from case 1: showing generalised irregular spike/sharp and slow activity in sleep without any clinical accompaniment
    - (B) Inter ictal EEG from case 6: showing short bursts of mixed spike and slow waves with a bilateral but asymmetric distribution
    - (C) Ictal EEG from case 6 during a focal-onset seizure: showing generalized spike and slow wave activity
    - (D) Inter ictal EEG from case 10: showing irregular spike/sharp and slow wave activity, more prominent in the left temporal and occipital regions
    - (E) Ictal EEG from case 10: showing right sided sharp waves and left sided slow waves
- Figure 2 – Facial appearances, from left to right: case 6, case 4, case 8, case 10

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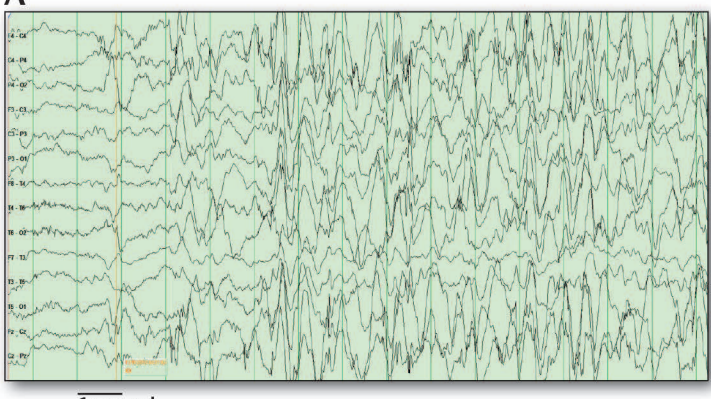

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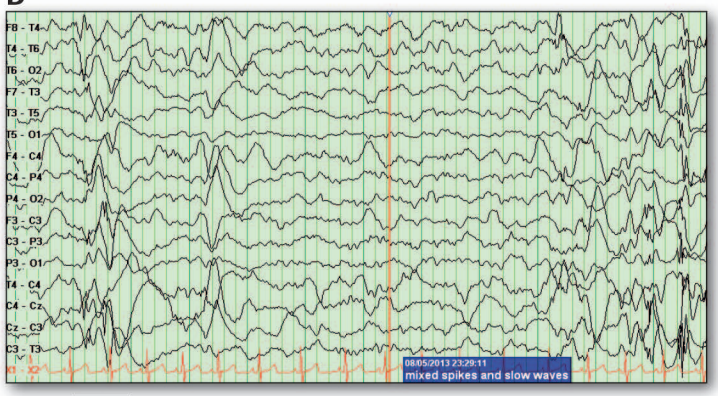

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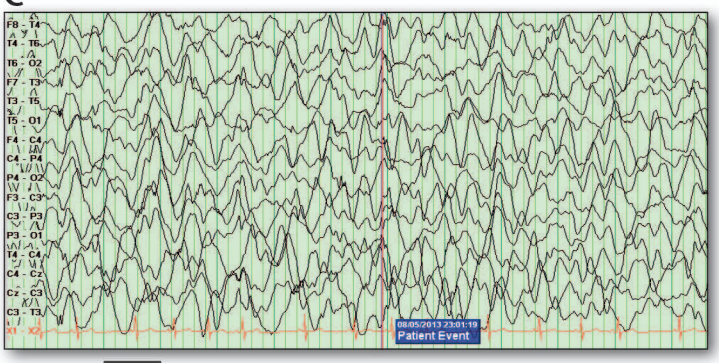

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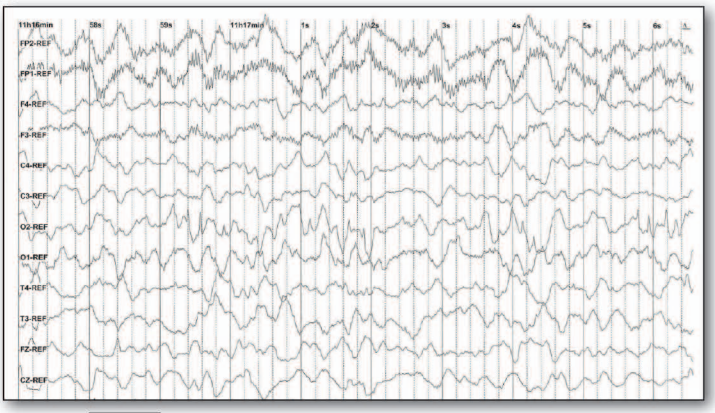

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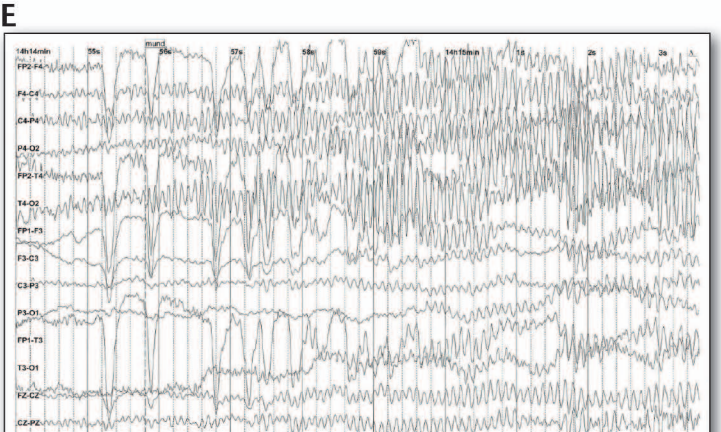

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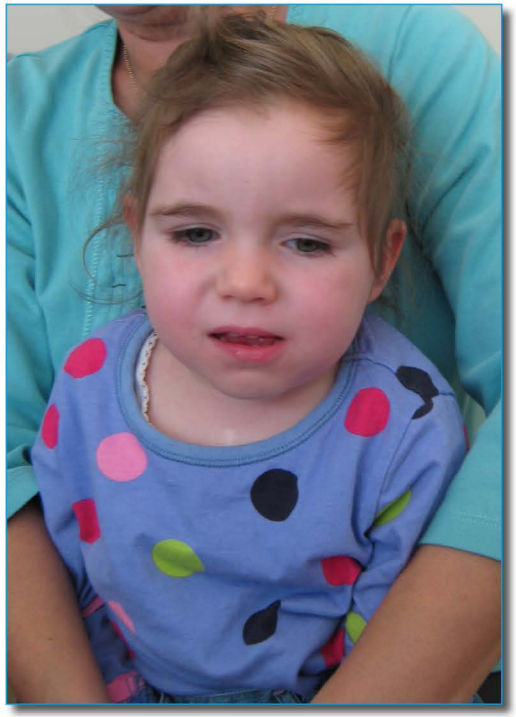

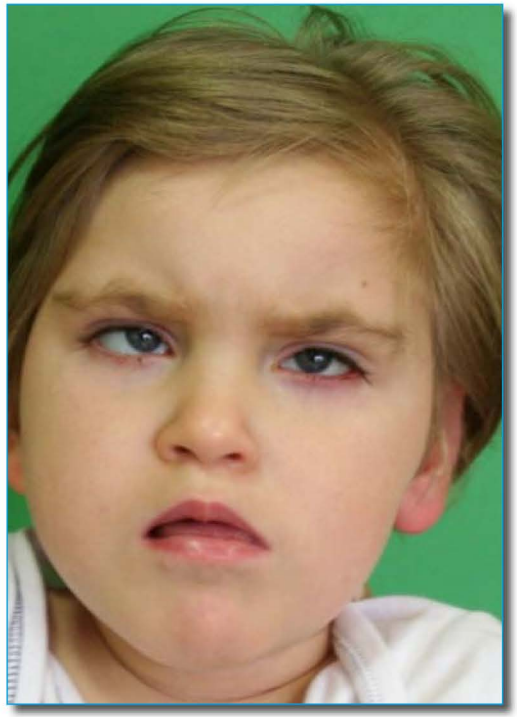
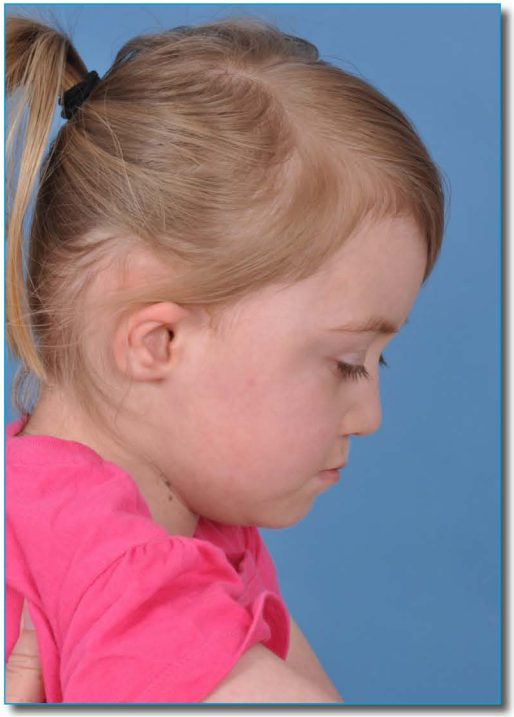


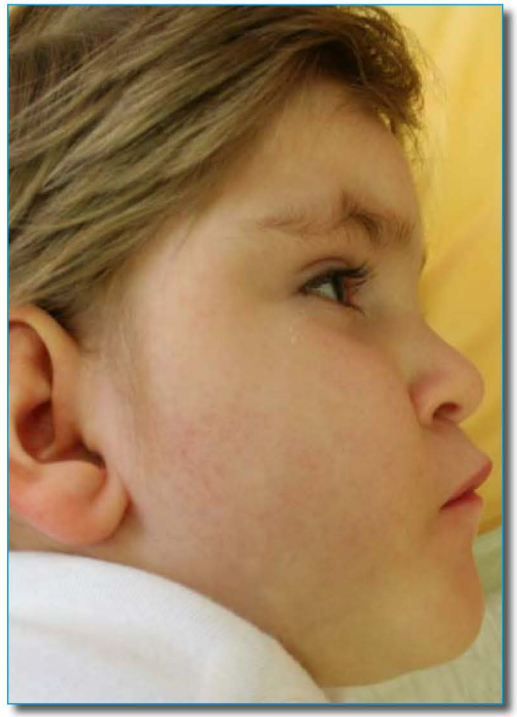

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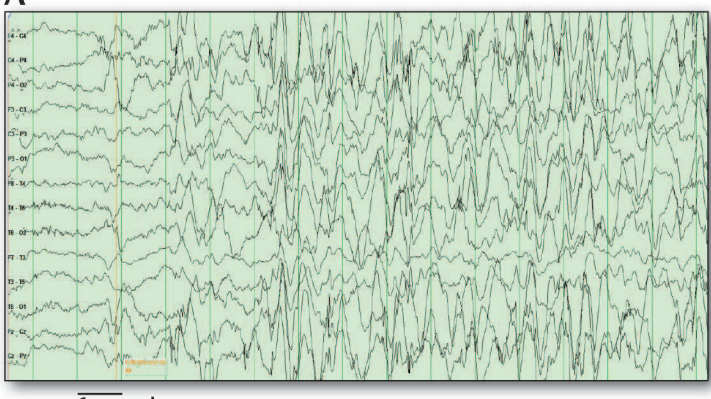

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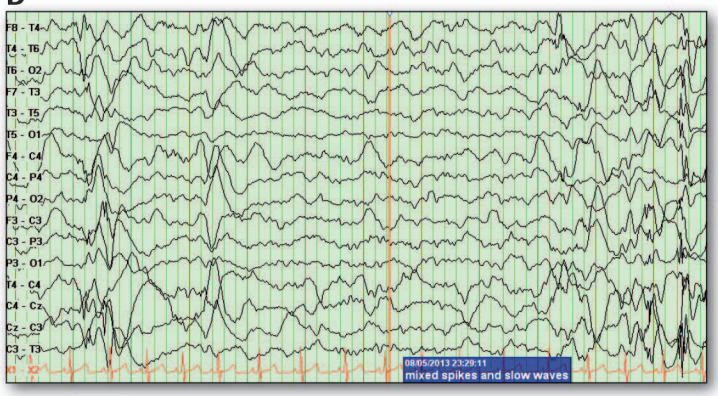

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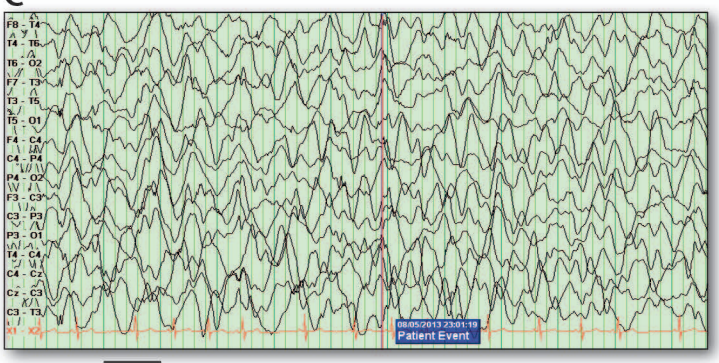

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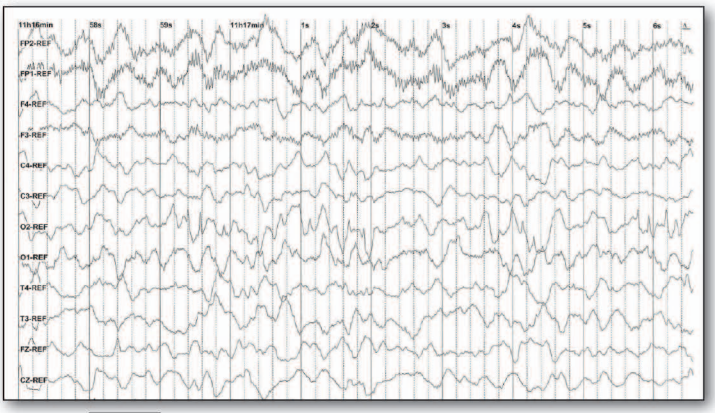

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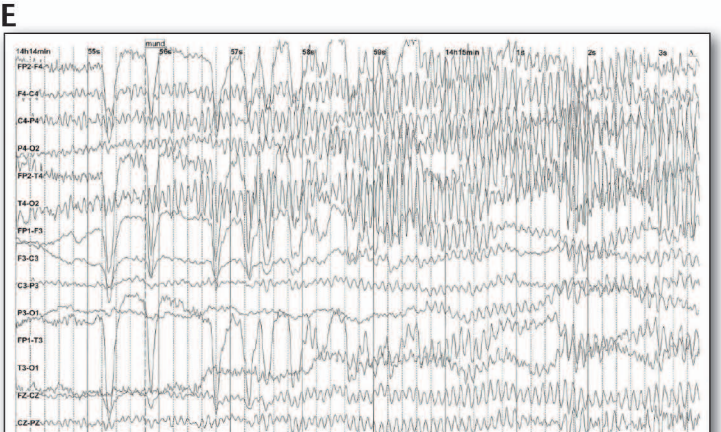

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Case	Base change	Amino acid change	Predicted effect on protein	Zygosity	Inheritance	X-inactivation studies
1	c.1591C>T	p.Gln531Ter	Truncation	Heterozygous	*De novo*	Moderately skewed
2	c.3145C>T	p.Arg1049Ter	Truncation	Heterozygous	*De novo*	Not done
3	c.549G>A	pGlu183Glu	Splice site interference	Heterozygous	*De novo*	Not done
4	c.2197G>T	p.Glu733Ter	Truncation	Heterozygous	*De novo*	Normal
5	c.3326_3330delATGGC insC	p.Asp1109AlafsTer102	Truncation	Heterozygous	*De novo*	Not done
6	c.2923C>T	p.Arg975Ter	Truncation	Heterozygous	*De novo*	Not done
7	c.511C>T	p.Arg171Ter	Truncation	Heterozygous	*De novo*	Normal
8	c.2477delA	Frameshift	Truncation	Heterozygous	*De novo*	Not done
9	c.2477delA	Frameshift	Truncation	Heterozygous	*De novo*	Not done
10	c.3115C>T	p.Gln1039Ter	Truncation	Heterozygous	*De novo*	76:24 ratio in blood

Table 1 - summary of genetic findings from our 10 cases

Subject 1

Subject 1 is a 6-year-old female. She was born at 34 weeks' gestation weighing 1420g (-1.8 SD), and with OFC of 29.5cm (-0.8 SD). There was established intrauterine growth restriction. Tone was normal for gestation at birth, but 3 weeks of feeding support via nasogastric tube were required. Gross motor delay became apparent at 6 months of age when she was unable to roll. She was first able to sit at 2 years of age, and she began walking at 2.5 years. She can now run with an unsteady gait, but she requires assistance to get to standing from sitting. She spoke her first words at 4 years. At one stage she had 6 words but has since lost them. She is sociable, happy and content. She makes hand gestures and responds to music with a smile. She eats pureed food only and is able to feed herself with a spoon.

She presented with a cluster of generalized tonic-clonic seizures (GTCS) at 15 months of age. On this occasion she had 5-6 seizures in a 3-day period. She was initially started on Phenytoin and Sodium Valproate and was seizure-free for 10 months. She now has clusters of nocturnal GTCS or hemiclonic seizures every 6 weeks with no obvious precipitant to clusters. She is currently taking Topiramate, Clobazam and Carbamazepine regularly. The hemiclonic seizures can affect either her left or right side. The following antiepileptic medications have all been tried but none have controlled seizures: Levetiracetam, Lamotrigine, Clobazam, Topiramate. 2 MRI brain scans have been normal. EEGs have shown independent left and right sided anterior epileptiform abnormalities, with sleep activation and runs of fairly generalised epileptic activity.

She has mild facial asymmetry with the right side of the face appearing fuller than the left. There is mild left ptosis and she has small hands and feet. Current height is 103.6cm (-2.3 SD), weight is 14.35kg (-3.0 SD), and occipito-frontal circumference (OFC) 48.5cm (-3.0 to -4.0 SD). She has low muscle tone and hypermobility.

Subject 2

Subject 2 is a 6-year-old female. She was born at T+12 weighing 3345g (-0.9 SD). OFC at birth was 33.7cm (-1.47 SD). At 4-5 months of age she was found to have significant hypotonia. She has severe developmental impairment. At the age of 6 years she is unable to sit independently, she has no meaningful use of her hands, and no verbal or non-verbal communication. She has had severe gastro-oesophgeal reflux, treated by gastrostomy and fundoplication. She has bilateral congenital hip dysplasia, bilateral talipes equinovarus, and a bifid T8 vertebra.

She presented with a seizure at 5-6 weeks of age. Initial seizures were described as tonic-clonic, but she went on to have focal seizures characterized by right facial drooping and cycling arm movements. Most seizures have been accompanied by apnoea. Seizures initially occurred every 2-3 days and were resistant to multiple therapeutic interventions, including Carbamazepine, Sodium Valproate, Topiramate, Clobazam, Rufinamide, Ketogenic Diet, and Vagal Nerve Stimulation. Levetiracetam made seizures markedly worse. She was started on Gabapentin at the age of 5 years 10 months and has been seizure-free since then. MRI brain at 4 months was normal, but at 5 years showed progressive cerebral volume loss. EEGs were initially normal, but more recently have been diffusely slow with sharp transients seen over both temporal regions.

She has marked bitemporal narrowing; upslanting palpebral fissures; fairly thin, straight eyebrows; slightly coarse facial features; mildly posteriorly rotated ears; mild tapering of her digits; slightly short 5th fingers; short broad halluces; and overlapping of the 2nd and 3rd toes. Most recent measurements aged 34 months were: length 83cm (-2.6 SD) weight 10.9kg (-2.1 SD), and OFC 40.8cm (-4.5 SD).

Subject 3

Subject 3 is a 3-year-old female. She was born at term weighing 3160g (-0.5 SD). OFC was not recorded at birth but was 38.5cm at 12 weeks of age (-1.6 SD). Following suction stimulation and oxygen for thick meconium at delivery, she was treated with incubator oxygen for the first 8 hours of life. A cleft palate was present at birth. There were neonatal feeding difficulties requiring nasogastric tube feeding. By day 35 of life she was bottle feeding by day, with overnight nasogastric feeds. She has severe global developmental delay. She rolled at 7 months, but will only occasionally do so. She is able to bring her hands together and clap, but is unable to hold anything in her hands. At aged 3 years she cannot sit without support and has poor head control. She has no words but can laugh, giggle and babble, and is socially responsive. She has cerebral visual impairment, fixing and following only fleetingly, and bilateral conductive hearing loss. She suffers with chronic constipation. Renal USS shows multiple small cysts.

Her first seizure was at 4 months of age and was characterised by bilateral upper limb stiffening, frothing of the mouth, and cyanosis. She has clusters of seizures which build up over several days and often require hospital admission for emergency management. Seizures have been refractory to Gabapentin, Acetazolamide, Clonazepam, Lorazepam, Sodium Valproate, Levitiracetam, Pyridoxine, and Phenobarbital. Introduction of Stiripentol was felt to improve seizure control. MRI brain showed a small hemorrhage along the posterior falx and tentorium thought possibly related to delivery. Repeat MRI scan showed normal myelination with diffuse reduced volume of the brain matter in the supra and infra tentorial levels. EEG shows high amplitude background activity and epileptiform discharges over left and occasionally right hemisphere.

She has truncal hypotonia with slightly increased limb tone, normal deep tendon reflexes, bilateral ankle clonus and downgoing plantar reflexes. She has hirsutism of the forehead, back, arms and upper lip, bitemporal narrowing with low anterior hairline, long eyelashes, and downturned corner of mouth with midline groove of the lower lip. Most recent measurements, at aged 40 months, are: height 97.9cm (0.06 SD), weight 14.8kg (-0.04SD) and (OFC is 47.3cm (-0.8 SD).

Subject 4

Subject 4 is an 8-year-old female. She was born at term weighing 3220g (-0.4 SD). OFC at birth was 32cm (-2.0 SD) and had dropped to below -2.0 SD by 8 months of age. She required nasogastric feeding support for the first 2 months of life. She has severe developmental impairment, with no verbal communication and no independent mobility at the age of 8 years. She has a diagnosis of autism spectrum disorder. She had an atrial septal and ventricular septal defect repaired in infancy.

Her first seizure was at 5 months of age. She had tonic posturing with head turning to the right followed by 4 limb clonic movements lasting 2 minutes. She continued to have similar seizures approximately every 2 days. She also developed generalised tonic seizures and seizures with unresponsive stares, considered most likely to be focal seizures. She had unprecipitated clusters of seizures. Skill loss was noted following seizure clusters. Her epilepsy was refractory to Sodium Valproate, Lamotrigine, Carbamazepine, Topiramate, Levetiracetam and Phenytoin. She had a period of control on Clobazam which then lost its effect. She has been seizure-free since commencing Phenobarbital. MRI brain is normal. Interictal EEG shows multifocal epileptic activity, ictal EEG has demonstrated right temporal lobe seizure-onset.

She has small widely spaced teeth, deep-set eyes, downward sloping palpebral fissures, and syndactyly of the 1st and 2nd toes. Most recent measurements were OFC 46.0cm at aged 49 months (2-3 SD below mean), height 74cm at aged 20 months (-2.6 SD) weight 7.9kg at aged 20 months (-4.0 SD). Examination reveals low tone with normal deep tendon reflexes.

Subject 5

Subject 5 is a 10-year-old female. A cerebral cystic lesion and abnormal positioning of the halluces were noted antenatally. She was born at term weighing 3005g (-0.9 SD). OFC at birth was 33cm (-1.2 SD) but fell to > 2.0 SD below the mean by 4 months of age. The cystic brain lesion was confirmed postnatally on MRI brain to be a cavum septum vergae. She has severe developmental impairment. She is unable to walk or to sit independently. She has no verbal communication and is unable to feed herself. She has cortical visual impairment. A gastrostomy was inserted at the age of 2 years. She has a bifid T6 vertebra, a small intra-atrial defect, and has been operated on for choanal atresia and stenosis which were contributing to episodes of stridor with cyanosis.

There was a plateauing of development prior to her presentation with seizures. She had clusters of eye twitching, limb-shaking, and impaired awareness. She presented with a cluster of 19 seizuresin a 24-hour period at the age of 6 months. EEGs showed continuous high voltage waves without epileptiform discharges. She subsequently developed myoclonic seizures, absence seizures, tonic seizures, epileptic spasms, and reflex seizures to sensory stimuli. She has had several episodes of non-convulsive status epilepticus and status myoclonus. Seizure clusters are precipitated by urinary tract infections. Seizures have been refractory to Phenobarbital, Carbamazepine, Clobazam, Sodium Valproate, Lamotrigine, Topiramate, Levetiracetam, and Pyridoxine,. Phenobarbital and Clobazam have been helpful as rescue therapy, whilst Paraldehyde and Clobazam have been helpful for seizure clusters. The use of the classical ketogenic diet reduced seizures significantly and allowed weaning of maintenance AEDs.

Subject 6

Subject 6 is a 5-year-old female. She was born at term weighing 2325g (-2.5 SD). OFC was -4.7 SD 8 weeks of age. Concerns about her visual behavior and lack of fine motor activity were noted at 6 months of age. She has global developmental delay. She was able to sit unsupported at 12 months and to walk at 30 months. She began babbling at 1-2 years of age but all verbal communication stopped following a 45-minute seizure at age 3 years.

Her first seizure was at 5 months of age. Her arms went stiff, with eyes deviated to the left, and loss of awareness. Subsequently she developed seizures with the same onset, but followed by clonic movements of all 4 limbs. She continued to have seizures like this, occurring in clusters of 3-10 seizures in a 24-hour period, every 10-21 days. Seizures have been refractory to Carbamazepine, Sodium Valproate, Topiramate, and Phenobarbital. She was seizure-free for 1 year on Levetiracetam before recurrence, and she had significant reduction in seizure frequency on the Ketogenic diet. MRI brain is normal. EEG demonstrates a high voltage slow background. Ictally there are bilateral bursts of high voltage slow waves with spikes and polyspikes intermixed.

She has puffy eyes; right nasal deviation; a small inturned right pinna; clinodactyly of the middle toes of both feet; and a central incisor. Current measurements are: height 97cm (-3.2 SD), weight 12.2kg (-4.2 SD), OFC (<-3 SD). Examination reveals generalized hypotonia with intact deep tendon reflexes.

Subject 7

Subject 7 is a 4-year-old female. She was born at term weighing 2722g (-1.5 SD). OFC was -1.3 SD. Her first seizure at 4 weeks' of age was a generalised seizure, characterised by clonic movements of all 4 limbs and the eyelids. She had had loose stools for a day before onset. Initial EEG was normal. By 6 months she was having tonic seizures sometimes in clusters and sometimes followed by a bilateral clonic phase. OFC was already on -2SD.The EEG then showed frequent multi spike and slow on the right centro-temporal region.

She has continued to have clusters of tonic-clonic seizures from September 2014, which did not respond to the addition of Topiramate, Valproate or Lamotrigine. Seizures can be unilateral right sided or left sided and have been followed by a right-sided Todd's paresis.

At 8 months she had poor muscle tone and gross motor delay. All developmental milestones have been delayed. At the age of 3 years 11 months she was just able to take a couple of steps holding onto a hand. She is sociable. She has no words but does coo, laugh, and cry appropriately. She shows limited social interaction and demonstrates no understanding of language with no meaningful communication gestures or words. Most recently she has lost interest in feeding despite having a safe swallow and requires PEG insertion. She is generally hypotonic but with increased dynamic tone at the ankle.

Measurements at 27 months of age were: height 78.8cm (- 2.5 SD), weight 10.8kg (-1.5 SD), and OFC 44.9cm (-2.0 SD). She has no dysmorphic features or malformations.

Subject 8

Subject 8 is a 14-year-old female. She was a twin born at 37 weeks' gestation. Birth weight was 2.324kg (-1.2 SD). She presented with delayed walking at > 2 years of age. She has severe developmental impairment and was diagnosed with autism at the age of 5 years. At the age of 7 years she had no verbal communication.

At 28 months of age she had a febrile convulsion. Following that she went on to have clusters of generalized tonic-clonic seizures 2-3 times per month, with 6-7 seizures in each cluster. Her seizures have been refractory to Phenobarbital, Levetiracetam, Topiramate, Lamotrigine, Carbamazepine, Clobazam, and Vagal Nerve Stimulation. MRI brain is normal. Sleep-deprived EEG shows right-sided spike and slow wave abnormalities.

Measurements at 6 years + 9 months of age were: height 102cm (-3.7 SD), weight 16.4kg (-2.4 SD), OFC 48.0cm (<-2.0 SD). She has no dysmorphic features or malformations.

Subject 9

Subject 9 is the twin sister of case 8. She was born at 37 weeks' gestation weighing 2000g (-2.0 SD). OFC at birth was 30.5cm (-1.7 SD). Multiple congenital anomalies were identifiable at birth including: hypotelorism; small low-set posteriorly rotated ears; bilateral 5^{th} finger clinodactyly; overlapping 4^{th} and 5^{th} fingers; left rockerbottom foot; and hypoplastic nails of the 4^{th} and 5^{th} toes. She had semilobarholoprosencephaly and partial anomalous pulmonary venous drainage. She had refractory neonatal seizures and she died at the age of 11 months.

Subject 10

Subject 10 died at the age of 9 years and 10 months. She was born at T+14 weighing 3185g (-1.2 SD). OFC at birth was 34cm (-1.3 SD). She had severe hypotonia that persisted throughout life. She was nasogastric tube fed from birth until 3 years of age when a gastrostomy tube was placed. She made very little developmental progress, was never able to sit, reach for objects, or communicate. She had atrial septal and ventricular septal defects repaired at 6 weeks' of age.

Her first seizure was at 2 months of age and was a generalized tonic-clonic seizure. These seizures continued to occur daily. From 6 months of age she developed myoclonic seizures occurring daily. She had episodes of status epilepticus 2-3 times per year. Seizures were refractory to Topiramate, Sodium Valproate, Leveiracetam, and Rufinamide. Serial MRI brain scans showed a thin abnormally shaped carpus callosum and minimal cerebral atrophy. Interictal EEG showed multifocal epileptogenic activity, more often over the right hemisphere.

Straight eyebrows and low set ears were noted at birth. Most recent measurements at 8 years and 6 months were: height 102.5cm (-5.0 SD), weight 16.1kg (-0.5 SD), OFC 43.7cm (-6.3 SD). She died of aspiration pneumonia.