Supplementary material

Patient Reports

Patient 1

This patient is a 3-year old female first child born to non-consanguineous, White European parents. Family history was notable for a paternal cousin with severe immunodeficiency who was identified as carrying a missense variant in the *BTK* gene, known to cause X-linked agammaglobulinaemia. Antenatal ultrasound at 20-weeks' gestation demonstrated Tetralogy of Fallot. The patient was born at term with a birth weight on the 50th centile. She required admission to the Neonatal Intensive Care Unit (NICU) due to her cardiac issues. She developed blue cyanotic spells necessitating corrective cardiac surgery at 3-months of age. She has a small residual ventricular septal defect.

She developed infantile spasms at 6-weeks of age. EEG (electroencephalogram) demonstrated hypsarrhythmia. A diagnosis of West syndrome was made. Magnetic Resonance Imaging (MRI) of the brain 7-months of age demonstrated asymmetry of the lateral ventricles and a thin corpus callosum. No focal neurological signs were evident on clinical examination, but significant developmental impairment was noted. She had partial response to the standard treatment of West syndrome, namely Vigabatrin and Prednisolone.

Her seizures subsequently evolved to myoclonic and tonic seizures in addition to ongoing spasms with clusters. These were refractory to several different treatment regimens including combinations of Vigabatrin, steroids, Sodium Valproate, Levetiracetam, and Topiramate.

She was started on classical ketogenic diet therapy at 12-months of age, together with cessation of all anti-epileptic medications, resulting in a partial response. At the age of 18-

months, she continued with clusters and single spasms through the day but with some improvement, and they were thought to be much less intrusive. By the age of 28-months, her spasms had decreased in frequency to two to three per day, as well as being of shorter duration, with some additional single myoclonic seizures. She had a course of ACTH (adrenocorticotropic hormone) therapy. This resulted in a definite, but short-lived, improvement in seizure control. Response to a second course of ACTH was less effective.

She has significant developmental impairment with limited head control and only occasional smiles and vocalisation, though these have improved with better seizure control. She was also noted to have shortening of her right lower limb, with a degree of hyperextension of the right knee. She was found to have severe cortical visual impairment, with no response to formal visual acuity assessment but some brief fixation. Electroretinogram (ERG) confirmed no evidence of diffuse retinal dysfunction.

On examination at the age of 11-months, her head circumference was on the 9th centile, length on 2nd centile and weight on 0.4th centile. She had brachycephaly, hypertelorism, bilateral low-set ears, bilateral proximally placed thumbs, an unusual symmetrical swelling of the medial aspect of both feet, right 2-3 toe syndactyly, and a proximally placed left second toe (Fig. 1). Radiological review of a skeletal survey demonstrated thoracic scoliosis, bilateral coxa valga and generalised osteopenia. There were unusual defects involving the right proximal and distal tibia, with the defect in the proximal tibia being larger. Overall, the right tibia was shorter than the left. Possible similar early changes were seen in the left distal tibia (Fig. 2). The significance of these radiological findings remains uncertain.

Chromosomal microarray analysis (CMA) demonstrated a 320 kb deletion at 6q26. The molecular karyotype was arr[hg19] 6q26(163310635_163630742)x1 pat. The deletion included one gene, *PACRG*, which does not have any significant disease associations. This deletion has also been seen in a number of healthy individuals and was inherited from a phenotypically normal father. It was therefore classified as likely benign.

Patient 2

This patient is an 8-years and 11-months old female, second child to healthy non-consanguineous, White European parents. Her 13½-year old brother had a single febrile seizure. Her father was 33-years old and her mother was 39-years old when she was conceived. She was born at 34^{+4} weeks gestation by normal delivery with a birth weight on the 25^{th}-50^{th} centile. She was admitted to the NICU on day five for excessive weight loss, jaundice, and skin ulcers. She was diagnosed with streptococcal skin infection and treated with intravenous antibiotics.

She developed infantile spasms at the age of 5-months. An EEG showed hypsarrhythmia and an MRI brain scan was normal. She was treated initially with Prednisolone and subsequently with Vigabatrin with partial response. Because of poor seizure control she was started on a ketogenic diet at the age of 21-months. Over the course of the next few years, she developed drop attacks, tonic spasms, and changes in visual attention associated with an unusual cry.

At the age of 4-years and 9-months she continued to have up to 30 seizures a day. Response to a variety of treatment regimens including Lamotrigine, Zonisamide, and Nitrazepam, was poor. Her seizure frequency, interestingly, reduced if she was febrile. Her developmental milestones were delayed. She could sit briefly without support and was

starting to weight bear with support. She had not developed any babbling sounds. There were no concerns about her eyesight and she had passed a hearing test. She had a gastrostomy to supplement her oral food intake and enable intake of fluids and medications. She had constipation that was treated with Movicol.
Her EEG at the age of 4-years and 3-months showed hypsarrythmia with frequent high-voltage, multifocal and generalized spike/sharp wave discharges with brief periods of relative suppression in the background.

Repeat MRI brain imaging at the ages of 18 and 31 months showed significantly delayed cerebral white matter myelination. This normalised by age 4-years, with some residual hyperintensity posterior to the trigones of the lateral ventricles. Magnetic resonance spectroscopy was normal. Baseline biochemistry, standard karyotype, CMA and *CDKL5* mutation analysis were normal.

She was reviewed in the Genetics clinic at the age of 8-years and 11-months. Her ketogenic diet had been stopped three months previously. Her antiepileptic therapy at this time comprised Rufinamide, Nitrazepam and Topiramate but she continued to have over 20 seizures a day, mostly drop attacks, absences and tonic spasms. She was able to walk a few steps with support. There were no concerns about her eyesight and hearing. She had excessive salivation that was treated with glycopyrronium. She had a MIC-KEY button in place which was used to give her the anticonvulsant medications. She could eat orally and drink using a straw. She was on chloral hydrate for her disturbed sleeping pattern. Her constipation had resolved.

On examination, her weight was just below the 0.4th centile, with height and head circumference significantly beneath the 0.4th centile. She had high-set eyebrows, long,

narrow palpebral fissures, mid-face hypoplasia with depressed nasal bridge, open mouth with full, tented upper lip and a protruding tongue (Fig. 1). There was a hypomelanotic patch over the left side of her neck anteriorly. Her hands were slender with long fingers and there was hyperlaxity at her knees. Her tone was normal with brisk knee jerks and an up-going right plantar response. Her spine was straight and there were no joint contractures.

A repeat MRI brain scan showed persisting T2 hyperintensity posterior to the trigones of the lateral ventricles. A pelvis x-ray showed bilateral coxa valga with partial hip subluxation (Fig.2).

Patient 3

This patient is an 8-year old female, first child born to non-consanguineous, White European parents. Family history was unremarkable. The patient was born at 43 weeks gestation with birth weight on the 25th centile and head circumference between the 98th-99.6th centiles. Early in infancy, she was noted to have social contact difficulties, hypotonia and abnormal development. She developed infantile spasms at 6-months of age. EEG demonstrated hypsarrhythmia. Her seizures responded to Vigabatrin in combination with ACTH. She was then treated with Valproate monotherapy. From the age of 3 ½ years, medication was withdrawn and she remained seizure-free. Neonatal nystagmus was noted, which ceased as seizure control improved. She had myopia, astigmatism, intermittent exotropia of the right eye and amblyopia ex anopsia. ERG performed at 14-months of age was normal.

At the age of 8-months, the following dysmorphic features were noted: almond-shaped eyes, epicanthus, hypertelorism, high-arched palate, thick and curly hair, inverted widely

spaced nipples and obesity. MRI brain and echocardiogram at this time were normal. CMA was normal.

On assessment at 2 ½-years, she had severe global developmental delay. She had severe hypotonia and could not sit independently, crawl or eat. She had no spoken language.

When reviewed at the age of 3 years and 4 months, she had severe hypotonia and global developmental delay, but with good eye contact and no spoken language. She had problems with reflux and constipation, and had central obesity. A few hypopigmented skin patches were noted. She had metopic ridging, elongated palpebral fissures, large, bright blue irises; blueish sclerae; slight eversion of the lower lids; long and prominent eyelashes; broad eyebrows; short nose with short, slightly overhanging columella and hypoplastic alae nasi; short, grooved philtrum; small mouth with a protrusion in the midline of the upper lip, posteriorly-rotated ears, tapering fingers, small hands and feet, prominent pads of the fingers and toes and broad great toes (Fig.1).

Kabuki syndrome was considered as a possible differential diagnosis, although her intellectual disability was more severe than typically seen in Kabuki patients, and sequencing of *KMT2D* (*MLL2*) and *KDM6A* was negative. *MECP2* analysis was also normal. A congenital disorder of glycosylation was suspected, but desialotransferrin and transferrin concentrations were normal, as were 7- and 8-dehydrocholesterol levels.

At the age of 8-years, she had severe intellectual disability with no spoken language. She can walk with support if helped to a standing position. She was less prone to infections than in early childhood. Because her height development was following the – 2 SD curve, she received growth hormone since 1-year of age with a partial response.

Patient 4

This 3-year old female patient is the fourth child born to non-consanguineous, White European parents. There was no significant family history. Increased nuchal thickening was noted on antenatal ultrasound. She was born at term with a birth weight on the 40th centile and head circumference on the 50th centile.

She developed infantile spasms at 6-weeks of age and EEG demonstrated hypsarrhythmia. A diagnosis of West syndrome was made. Her spasms were partially controlled with a combination of Vigabatrin, steroids and ACTH therapy. She was treated unsuccessfully with Phenobarbital, Valproate, Levetiracetam and Topiramate. Her spasms ceased with a combination of Vigabatrin, Nitrazepam and ketogenic diet at 2-years and 3-months of age.

On examination at age of 3-years and 4-months, she had global hypotonia. She could sit and stand with help. She had no spoken language. She had severe intellectual disability. Her head circumference was on the 40th centile, length on 75th centile and weight on the 50th centile. Dysmorphic features included brachycephaly and plagiocephaly, high forehead with low anterior hairline and a frontal cutaneous angioma. She had camptodactyly of the second right finger. There were no ophthalmic abnormalities.

MRI brain scan at two months was normal. A second MRI brain at 2 ½ years of age demonstrated thinning of the corpus callosum with delayed myelination and slight enlargement of the lateral ventricles. Western-blotting of serum glycoproteins did not show abnormal isoforms of transferrin or orosomucoid.

Patient 5

This 10-month-old female patient was born to non-consanguineous White European parents. The pregnancy was unremarkable. She was born at 38 weeks gestation with a birth weight between the 75th and 91st centiles. She was admitted to the Neonatal Intensive Care Unit for four days as she required continuous positive airway pressure support.

She developed episodes of eye rolling and chewing at the age of 4-months. She additionally had occasional twitching of her leg and myoclonic jerks affecting one arm. Epilepsy was suspected. At 5-months age, EEG demonstrated focalised epileptic activity. Carbamazepine was started with some initial reduction in the eye rolling episodes. Levetiracetam monotherapy was then trialled with minimal effect. However, EEG at 9-months did not demonstrate any definite ictal changes. As there was uncertainty regarding the correlation of EEG changes and possible seizures, anti-epileptic medication was stopped with no clinical change.

On examination at the age of 10-months, she had moderate to severe global developmental delay. She had hypotonia. Dysmorphic features included epicanthus, broad nasal bridge, and prominent broad forehead. Her head circumference was on the 50th centile, weight 91st - 98th centiles and height 91st centile.

She had reduced eye contact, with unsteady eye movements and strabismus. Ophthalmological examination was otherwise normal. MRI brain scan was normal, as were a radiograph of the thorax and ultrasound of the hips.
Standard testing for Spinal Muscular Atrophy, Myotonic Dystrophy and Prader Willi syndrome was normal.

As well as the variant in *SLC35A2,* exome sequencing demonstrated variants in *ERMARD* c.1336A>C;p.(Ser446Arg) and *SCN2A* c.4169T>C;p.(Ile1390Thr). The *ERMARD* variant is a variant of uncertain significance according to ACMG criteria, however the phenotype associated with this gene (periventricular nodular heterotopia) was not present in the patient. The *SCN2A* variant is likely benign according to ACMG criteria.

Methods

Patient 1

The patient was ascertained through routine referral to the local Clinical Genetics service, with a referral indication of refractory epileptic encephalopathy with normal brain imaging. CMA was performed using an OGT 60K v2.0 ISCA oligo array and data analysed using CytoSure Interpret Software v4.5.3. Sequencing of 72 genes associated with early infantile epileptic encephalopathy was carried out using targeted whole exome sequencing (WES) (Agilent SureSelect and MiSeq) with 99.9% of the coding bases in the targeted genes covered to a depth of >30x.

Patient 2

The patient was also ascertained through routine referral to the local Clinical Genetics service, with a referral indication of refractory epileptic encephalopathy. CMA was performed using an ISCA 60K oligo array and data analysed using BlueFuse Multi software (v2.5). Sequencing of 72 genes associated with early infantile epileptic encephalopathy was carried out as above.

Patient 3

CMA was performed using Affymetrix GeneChip 250K Nsp, followed by trio-based whole genome sequencing (WGS). Libraries were prepared using Illumina's PCR-free protocol (TruSeq DNA Sample prep kit –Illumina). Samples were sequenced on Illumina HiSeqX system (Illumina) and v2 sequencing chemistry to at least 30x coverage using standard protocols. BWA was used to map sequence reads to human reference genome using default settings. Variants were called using Genome Analysis Toolkit (GATK) and the standard GATK workflow (Broad Institute).

Patient 4

The patient was also ascertained through routine referral to the local Clinical Genetics service, with a referral indication of refractory epileptic encephalopathy. CMA was performed as previously described, with an 180,000-oligonucleotide (180K) microarray (Sure Print G3 Human CGH Microarray Kit, Agilent Technologies). Sequencing of a 93 gene panel for monogenic epileptic disorders was performed on a NextSeq500 (Illumina) after sonication of genomic DNA (Covaris) and library-building with SeqCap EZ (Roche). DNA was treated with the EpiTect Bisulfite kit (Qiagen) according to the manufacturer recommendations. X-inactivation was studied by semi-quantitative PCR on the FMR1 and Androgen Receptor (AR) loci, using in-house protocol. PCR fragments were run on a CEQ-2000XLS (Beckman Coulter) and ratios were calculated for each locus as the average of ratio for the methylated alleles and ratio for unmethylated alleles, as long as the locus was informative.

Patient 5

CMA was performed as previously described. WES was performed using NovaSeq 6000 Agilent SureSelect All Human Exon v6.

All data was analysed against GRCh37. All variants reported are according to the NM_005660.2 transcript. Variants were verified by Sanger sequencing using standard protocols.

Supplemental Figure Legends

Figure 1. Brain MRI images of Patient 1 at age 7 months. Figure 1a shows T2 weighted axial image showing myelination of anterior and posterior limb of internal capsule, asymmetry of ventricles and thin corpus callosum. Figure 1b shows T1-weighted sagittal image through midline showing thin corpus callosum (white arrow). Figure 1c shows coronal FLAIR image through temporal lobes showing normal hippocampal morphology (white arrows)

Figure 2. Serial brain MRI images of Patient 2 at different ages. Figures 2a-e: Axial T2-weighted sections at age 18 months, 31 months, 4 years, 7 years and 9 years respectively. Figures 2a and 2b show significantly delayed cerebral white matter myelination. Figure 2c shows relative normalisation of the appearances of the cerebral white matter with residual hyperintensity posterior to the trigones of the lateral ventricles (white arrows), which remains unchanged in Figure 2d and e (white arrows). Figure 2f: reconstructed coronal T1-weighted image through temporal lobes at age 4 years showing abnormal morphology of the hippocampi, which appear small and malrotated (white arrows).