The American Journal of Human Genetics, Volume *108*

Supplemental Data

Missense variants in the N-terminal domain of the A isoform of FHF2/FGF13 cause an X-linked developmental and epileptic encephalopathy

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Supplemental Note: Case Reports

Individual 1

This male individual is the second child of unrelated White British parents. He has an older sister who is healthy and a younger sister (individual 2) who also has infantile-onset epilepsy and developmental delay. He was delivered by ventouse at full term following an uncomplicated pregnancy. His birth weight was 2.83 kg (-1.5 SD). In the first 24 hours of life he was noted to be restless and had four episodes of apnoea, cyanosis and lip smacking following feeds. His stomach and oesophagus were washed out to remove excess mucus. At 11 days of age he had another episode of apnoea and cyanosis. He was resuscitated and taken to hospital. Gastroesophageal reflux was suspected based on the results of a barium swallow.

At 4 months of age individual 1 had further events associated with staring, twitching of the right eye and posturing of his right arm. An MRI brain scan was normal, and an ictal electroencephalogram (EEG) showed sharp waves over his left temporal region. He had his first episode of convulsive status epilepticus at 7 months of age. He went on to develop slowly with no period of regression. At 8 months of age he could roll over with prompting but could not sit without support. He could reach out for objects directly in front of him. His seizures were characterised by staring, vomiting and lip twitching. Events lasted 10-20 seconds but occasionally up to 1 minute. His sleep was sometimes fragmented, with frequent episodes of inappropriate laughing or giggling. These were thought to be ictal.

By 9 months of age the seizures had evolved to start with a gag or vomit, and excess salivation. He would then swallow, his eyes would deviate to the right, and his right eye would twitch; they would end with a ‘nervous’ laugh. In some cases, he would hold his breath, become cyanosed and have a generalised seizure. On examination, his occipitofrontal circumference (OFC) was 44.5 cm (-1.5 SD). There was no overt laterality. His tone was slightly low. He was relatively inactive in terms of gross motor movements when placed prone or supine. He could, however, raise his head and upper trunk.

A further EEG at 9 months of age showed an asymmetric record with almost continuous discharge from the right temporal leads. The background amplitude was slightly higher than expected for his age. A diagnosis of epilepsy of infancy with migrating focal seizures was considered. However, the EEG findings were not felt to be consistent with this classification as there was no shifting of foci of ictal onset between hemispheres during the same EEG recording.

Individual 1 had a seizure-free period around 10-12 months of age (on lamotrigine, topiramate, vigabatrin and sodium valproate). Towards the end of this period the vigabatrin was stopped. This coincided with him becoming more alert and responsive. At 12 months of age he was more visually attentive and became more interested in toys but was not reaching out for them. He could stand with support. OFC at 12 months was 45.5 cm (-1.8 SD). His muscle tone continued to be slightly low. Eye examination at 13 months revealed symmetrical corneal reflexes with no nystagmus. His fundi were normal. He had slight astigmatism but it did not require correction. At 15 months of age he had 2 prolonged seizures, the first was predominantly left sided and lasted 45 minutes, and the second was predominantly right sided and lasted 30 minutes. This was in the context of him having an unspecified (possibly viral) illness.

On review at 20 months of age individual 1 was having around 3 seizures per week (this was after stopping lamotrigine). These typically occurred early in the morning and lasted for about a minute. He would initially vomit or gag, following which his head and eyes would deviate to the right. His right eye would twitch followed by twitching of his right arm. On examination, his tone was normal although his feet were still quite mobile and his back was quite rounded. OFC at 22 months was 46 cm (-3 SD).

A ketogenic diet was employed between 20-23 months of age however individual 1's seizure frequency increased. This coincided with a change of seizure morphology to include truncal flexion at the start of seizures. He would then start with focal involvement on the right which would spread to his face, with chewing. There was an element of retained awareness during these events.

At 24 months of age individual 1 was having about 25 seizures per month. The seizures were generally short and stereotyped. He would initially flex his trunk, followed by trembling and clawing at his face. He would then relax slightly, followed by a gustatory component (swallowing, chewing, jaw movement and vocalisation). Examination showed profuse drooling, low axial muscle tone and occasional athetoid and dystonic movements. A 24-hour EEG at 2 years of age captured a seizure during sleep. At onset there was diffuse alpha activity followed by rhythmical sharpened slow wave activity in the right posterior hemisphere leads, thereafter with diffuse slowing over both hemispheres. Interictal epileptiform activity was seen over the right posterior hemisphere. Clinically, the event lasted 4 minutes and manifested as deep breathing, with oromotor automatisms. There was an ictal tachycardia.

At 29 months of age individual 1 was commando crawling and was aware of bottles put into his hands. His sleep pattern was still poor and he was treated with melatonin. The frequency of his focal seizures ranged from daily clusters to single isolated seizures once a week. The typical duration was 1-2 minutes but sometimes up to 3 minutes. Seizures involved head deviation to the left, eye twitching and repetitive swallowing. Both arms would flex but towards the left side of his body. He would then have a clonic phase and becoming dusky around the mouth. Events were usually nocturnal and he would go back to sleep afterwards. At 2½ years of age a vagus nerve stimulator (VNS) was fitted. This improved recovery from seizures but not the frequency. Individual 1 sat unaided at 4 years of age.

At 5 years of age individual 1 was not playing with toys but liked lights and to tap objects. He had a good appetite and fed orally. He could chew well but continued drooling. On examination, he had deep-set eyes but was not dysmorphic. He was well grown and had normal hand creases. His neurology was normal. OFC was 50 cm (-1.9 SD). Constipation was a persistent problem for individual 1 from infancy onwards. He had increasing problems with abdominal discomfort around the age of 7 years. Blood tests for celiac disease and thyroid function were normal. An EEG at age 8 years (on vigabatrin, sodium valproate, topiramate and oxcarbazepine) showed generalised spike-wave activity throughout.

At 8 years of age a barium meal and follow through concluded that he was grossly constipated, with poor colonic motility despite large doses of bulk-forming agents and bowel stimulants. At 9 years 10 months of age he underwent elective sub-total colectomy and ileostomy. However, the abdominal pain continued and at 11.5 years of age he had a laparotomy to divide adhesions and to release a stoma constriction. Post-operatively his weight was 36.4 kg (+0.0 SD) and he was feeding well orally. The revised stoma functioned well without difficulties. Despite the operations individual 1's abdominal pain continued in paroxysms every 7-9 days. Visceral hyperalgesia was raised as a possible explanation although the discomfort seemed to respond to an anti-spasmodic agent. His focal seizures continued during this period. Other seizure types included drop attacks and gelastic seizures in the early hours of the morning.

When reviewed at 13 years of age individual 1 could take his own weight when standing but was not walking independently. His parents felt his concentration had improved over the previous two years. Daily routines were important for him. He was not talking or pointing but had responsive eye gaze and vocalised when he wanted something. He would occasionally comfort rock, hand flap or hyperventilate. Examination at this stage showed that his weight was 55kg (+0.7 SD) and OFC 53.4 cm (-1.4 SD). He had mild scoliosis and gum hypertrophy. Neurological examination found low truncal tone, with tight hamstrings but no lateralising signs.

Individual 1 continued to have a range of seizure types. He had daily focal seizures and weekly generalised tonic-clonic seizures (GTCS). The focal seizures were short, typically 1-2 minutes. They were often associated with repeated swallowing or gagging, breath holding, and increased salivation. His arms would tighten and then start jerking. His head would turn to the right (or sometimes the left). He would go pale and his lips might go blue. Some events would begin with his throwing back his head, splaying out his arms, and shouting as if frightened. He would be happy with intermittent giggling afterwards, with mouth and eye twitching for a few minutes. With generalization the focal component would be followed by him going blank and extensor posturing. An asymmetric clonic phase would then follow, with his arms and legs jerking, particularly on the right. His face would become red and blotchy. These seizures were occasionally prolonged (10-90 minutes long). These were characterised by jaw, eye and limb twitching, and being very unresponsive. Sometimes he would vomit. His anticonvulsant therapy

included sodium valproate, phenobarbitone, gabapentin, zonisamide and buccal midazolam (as required).

Individual 1's genetic testing has included routine karyotype, array comparative genomic hybridization (aCGH), single gene testing for Fragile X syndrome, *CDKL5*, *SCN1A* (including dosage analysis) and a 134-gene infantile epilepsy panel. The results were all normal.

Summary of antiepileptic medications (Figure S2, current anticonvulsant drugs underlined):

Vigabatrin 4m(onths) – 11m ("alert and more responsive after coming off"), 26m – 113m
Sodium valproate 4m – 18m, 33m – 36m, 106m +
Lamotrigine 4m – 18m, 108m +
Topiramate 9m – 110m
Levetiracetam 18m – 24m
Oxcarbazepine 36m – 107m
Pregabalin 48m – 49m
Rufinamide 56m ("many jerks"), 105m ("made seizures worse", "drugged up"), 108m – 149m
Sulthiame 63m – 72m ("less seizures after coming off")
Clobazam 100m – 106m
Phenobarbital 107m, 113m – 173m
Gabapentin 154m +
Zonisamide 157m +
Perampanel 173m +

Individual 2

This female individual is the third child of White British parents. She is the younger sister of individual 1. She was born by elective caesarean section at 39 weeks gestation following a uneventful pregnancy and required no special care. Cord pH was normal. Apgar scores were 9 at 1 minute and 10 at 5 minutes. Her biometry at birth was OFC 33 cm (-0.8 SD) and weight 3.13 kg (-0.2 SD). At 11 days of age she presented with several episodes of becoming dusky around

feeds and shallow breathing. She had a respiratory arrest. She was resuscitated, admitted to the Paediatric Intensive Care Unit and given a loading dose of phenobarbital. The apnoeic events stopped and she was discharged from hospital after 9 days. Gastroesophageal reflux was suspected and she was not given further anticonvulsant drugs at this stage. An interictal EEG at age 1 month was normal with no photosensitivity.

When reviewed at 7 weeks of age she was feeding and growing well. Her OFC was 37.5 cm (+0.5 SD) and weight 4.65 kg (+0.8 SD). She was smiling responsively and had good head control. Limb tone and movement were normal. Her parents thought she was able to do more than her brother at the same age. She sat unsupported at 7 months of age. An interictal EEG at 10 months of age was normal apart from a reduction of sleep spindle and beta activity over the left hemisphere. There was no photosensitivity. At 11 months of age individual 2 had an upper respiratory tract infection and started to have focal seizures. The seizures were similar to but less severe than her brother's. The seizures involved lip smacking, going dusky around the mouth, repeated swallowing movements, eye deviation to left, and facial twitching on the left side. There was no generalisation. Her development and neurological examination was normal.

Individual 2 walked unsupported at 15 months of age. An interictal EEG at 14 months found excess background slowing. Ophthalmology examination at 19 months of age was normal. A cranial MRI at 20 months of age was normal. By 21 months of age she was having clusters of seizures which lasted for about 2 days every 6 weeks. Her parents began to notice some slowing of her development compared to her peers. An EEG at 23 months (on sodium valproate and vigabatrin) showed diffuse fast activity in the majority of leads with some non-specific mixed slow waves over the central and posterior regions. No epileptiform activity was noted and there was no photosensitivity. At 24 months of age individual 2 was walking and running, but her gait was still immature. She had difficulty standing from sitting and had to pull herself up on furniture. She could finger feed and use a spoon. She was noted to have speech and language delay and drooled profusely. She said ‘dada’ and ‘mama’ but not consistently. She had recurrent episodes of otitis media and at 28 months she was diagnosed with bilateral middle ear effusions. The seizures continued and were often nocturnal.

Reviewed at 31 months she had mild developmental delay, no speech and drooled profusely. Her growth was normal and she had good eye contact. Her seizures were well controlled on vigabatrin and topiramate but her parents reported general cognitive decline. Her development was better than her brother and she had less seizure activity. At 3 years of age her motor skills were better than her language abilities. She could use a pincer grip and was precise with buttons. She could climb stairs while holding hands, drink from a feeder cup, smile and vocalise. On examination her OFC was 48 cm (-2.1 SD). She was not dysmorphic but had a high palate and was slightly prognathic.

At 3½ years of age her seizures increased in frequency. The seizures were focal and typically involve her gulping, gagging or vomiting. Her lips would go blue. She would then have eyes deviation to the left with mouth twitching. The seizures were short and she would recover quickly from them. A short interictal EEG at 3 years 6 months of age (on lamotrigine, levetiracetam and vigabatrin) found diffuse fast activity overlying slower rhythms but no focal events. At 3 years 8 months of age she had a generalised seizure at home which was similar in morphology to her brother's.

At 4 years of age she was having frequent short focal seizures sometimes as clusters and sometimes as single isolated events. Her seizures were associated with eye deviation to the left (or sometimes right), lips going blue, and often ictal vomiting. They did not generalise. Her weight was 18.41 kg (+1.0 SD) and height 105.4 cm (+1.0 SD). EEG at 4 years of age showed diffuse fast activity mixed with non-specific slow wave activity but no definite epileptiform activity. Around 4½ to 5½ years of age she had a prolonged seizure-free period of several months on vigabatrin, sodium valproate and lamotrigine (after switching from vigabatrin, lamotrigine, phenobarbital and phenytoin). The mild developmental delay persisted. She was noted to have limited social communication. She was friendly but her behaviour was often challenging. Eye examination at 5 years of age was normal.

At 6 years of age individual 2 was having clusters of seizures every 5 or 6 weeks. She had a pervasive developmental behaviour pattern in contrast to her brother who had profound developmental delay. Her seizures typically had a left-sided focal onset, occasionally with

generalization. Her seizures were usually accompanied by vomiting and cyanosis. On examination, her neurology was normal. Her gait stability had improved. OFC was 53.5 cm (+0.8 SD) and weight 23.48 kg (+0.3 SD).

At 7 years of age individual 2's social and communication skills were assessed to be at the level of 1 year 2 months while her motor skills were equivalent to 1 year 9 months. Autistic features were noted. Her height was 121.6 cm (+0.1 SD) and weight 27 kg (+1.0 SD). She had been seizure free for several months on lamotrigine and sodium valproate but then began having clusters of seizures again. Repeat MRI brain found atrophic changes particularly affecting the left parietal and occipital regions. An ictal EEG suggested a left fronto-termporal origin. Fluorodeoxyglucose positron emission tomography (FDG PET) found reduced uptake of tracer in the mesial and anterior temporal lobe on the left. Uptake elsewhere was normal. Individual 2 underwent left anterior temporal lobectomy and partial amygdalo-hippocamptectomy at the age of 7 years 7 months of age.

At 8 years 9 months of age individual 2's social and communication skills were at the level of 1 year 6 months and motor skills at the level of 22-24 months. Her height was 132.8 cm (+0.2 SD) and weight 27.4 cm (-0.2 SD). EEG at 8 years and 10 months of age (on sodium valproate and lamotrigine) found only isolated sharp waves with phase reversal over the left hemisphere. There was no photosensitivity. Her seizures were well controlled at this stage and the lamotrigine was discontinued at 8 years and 11 months of age.

At 9 years of age she was being educated in a special needs school. She was described as having a pervasive developmental disorder. Eye examination was normal with a minimal degree of hypermetropia. The brain surgery had helped for 2 years but attempts to wean her sodium valproate coincided with a recurrence of seizures. During seizures individual 2 would frown, become slightly confused, gag and sometimes vomit. Her right eye would start to twitch and this would often continue throughout the seizure. Her right arm and right leg would become weak and she would fall. She would become blue around the mouth, repeatedly swallow and smack her lips. Her whole body would jerk repeatedly. Seizures would occur at night or when she was unwell. Her weight at 10 years of age was 36.8 kg (+0.7 SD).

A review when she was 11½ years of age showed that individual 2 was able to use about 30 words and sing nursery rhymes. She was noted to have autistic features. Routines were important for her but she had good eye contact. She would lead people to objects she wanted. She was affectionate but often became frustrated and aggressive which was challenging for her family. There were no concerns about her vision or hearing. She was having occasional seizures associated with lip smacking, swallowing, asymmetric limb jerking (more on right side), with the event ending with her screaming. Neurological examination was normal.

Individual 2's genetic tests have included aCGH, single gene testing for *ARX* and *FOXG1*, and a 30-gene infantile epilepsy panel. The results were all normal. Her other investigations have included a full suite of blood, urine and CSF investigations. The results were all normal. Echocardiography at 1 month of age found a tiny patent foramen ovale. Renal scan at 2 years 3 months of age was normal.

Summary of antiepileptic medications (Figure S3, current anticonvulsant drugs underlined):

Sodium valproate	11-26 m(onths), 55m+ (long seizure-free period after re-introduction)
Oxcarbazepine	14 m, 50m (no response, possibly more seizures on withdrawal)
Clobazam	Intermittent (no response)
Phenobarbital	Infancy, 52 m (irritable after loading)/
Vigabatrin	17m - 72m (initially reduced seizures frequency, difficult to wean)
Levetiracetam	21m -25m, 39m-43m (some response to increased dose, better developmental progress after coming off)
Topiramate	26m -33m (good control with vigabatrin and high dose topiramate, but parents reported cognitive impairment)
Lamotrigine	31m - 108m, 155m+ (stopped after epilepsy surgery but recently restarted)
Ethosuximide	46m -49m (no response)
Phenytoin	54m -55m
Lacosamide	67m - 70m (very sleepy)
Rufinamide	70m

Eslicarbazepine	135m ("not really successful")
Perampanel	147m - 151m (no seizures but behaviour deteriorated)
Pregabalin	151m

Individual 3

This male individual (Decipher ID 260211) is the first child of unrelated White British parents. He has two younger brothers, the youngest of whom (individual 4) also has early-onset epilepsy and developmental delay. Echogenic intracardiac foci were noted at 20 weeks gestation. He was delivered by ventouse following a prolonged labour at 39 weeks gestation. His birth weight was 3.4 kg (+0.1 SD). He did not require special care and was discharged home at 2 days of age. He presented at 1 month of age with persistent sleepiness and poor feeding. He had a septic screen including a lumbar puncture. It was noted on the ward that he was having recurrent apnoea (episodes of oxygen desaturation to 49%) and demonstrated some jerks and tonic posturing. It was concluded he was having seizures. He was sedated and ventilated. He was discharged home after a few days.

At 2 months of age individual 3 had further episodes of hand stiffness and grunting. He was diagnosed with focal seizures. The seizure morphology evolved to include brief tonic spasms. By 6 months of age he was having full flexor spasms. EEG confirmed hypsarrhythmia. Treatment with steroids and vigabatrin did not help. Individual 3 had global developmental delay and sat at 2 years of age. He subsequently developed severe scoliosis.

Individual 3 had a tonsillectomy at 5 years of age. He was noted to have an intermittent squint at 7 years. He was educated in a special needs school. Puberty started early, at 9-10 years, with hair development. He had an orchidopexy at 14 years of age. Brain MRI was reported to be normal. EEG showed a slow spike wave background consistent with Lennox–Gastaut syndrome.

Individual 3 was last reviewed at 19 years of age. He had severe learning disability, cortical visual impairment, and autism spectrum disorder. He could walk short distances with a frame. He tends to kneel-sit and could move around the floor in that position. He was very sensitive to

touch sensations. Individual 3 was non-verbal but vocalised and seemed to understand things that were said to him. He laughed when spoken to. He reached out for objects. He fed on soft solid food by mouth but required one-to-one feeding. He had severe gastroesophageal reflux disease. There had been three episodes of pneumonia over the previous two years. Individual 3 had periods of oral refusal possibly due to seizure activity or increased distress through constipation, leading to severe dehydration. Gastrostomy was being considered. He was not yet toilet trained. He was prone to chronic constipation. This required enemas and large doses of Movicol and washouts. Individual 3 also had hay fever and an urticarial reaction to peanuts.

Individual 3 had not had periods of regression or episodes of non-convulsive status epilepticus (NCSE) like in his brother. He had a VNS implanted. His seizure types included frequent brief absences with lip quivering (e.g. 1-2 per hour), GTCS, head drops associated with colour change and then anger, and previous gelastic seizures. Individual 3 had frequent myocolonic jerks during sleep often associated with grunting. Fever did not increase seizures frequency. His seizures were less of a problem when ill. On examination, individual 3 had limited eye contact, low truncal tone, no tremor, gum hypertrophy, and scoliosis. His hand creases were normal. He had 4 café au lait marks including a prominent one (~8 cm) on his back. His weight was 68 kg (-0.1 SD) and OFC 57 cm (-0.2 SD).

Individual 3's genetic tests have included aCGH, and single gene testing for *ARX* and *MECP2*.

Summary of antiepileptic medications (current anticonvulsant drugs underlined): <u>phenytoin</u>, vigabatrin, <u>lacosamide</u>, <u>clobazam</u>, <u>clonazepam</u>, cannabidiol, perampanel (caused agitation), sodium valproate, levetiracetam (on 2-3 occasions), carbamazepine, topiramate, rufinamide, zonisamide, brivaracetam and buccal <u>midazolam</u> (as rescue medication).

<u>Individual 4</u>

This male individual (Decipher ID 260212) is the third child of White British parents. He is the youngest brother of individual 3. Left-sided renal pelvis dilatation was diagnosed antenatally. He was born by elective caesarean section at 38 weeks gestation. He struggled with abdominal

pains, constipation, and gastroesophageal reflux as a baby but these improved over time. He presented with seizures at 6 months of age. These were described as brief focal seizures with his head and neck turning usually to the right with a clicking noise or licking of his lips. He sat at 11 months and walked at 15 months. Concerns mainly revolved around his communication from early on as he was never verbal.

Treatment with levetiracetam was started after one year of age and he was well controlled on this until 2½ years of age when valproate was added. At 2½ years of age he had a cluster of seizures associated with pyelonephritis and respiratory syncytial virus infection. The seizures were associated with vomiting, apnoea and cyanosis. The seizures were terminated with intravenous phenytoin and he was then put on maintenance phenytoin. Following this he developed NCSE and was noted to have epileptic spasms. His development regressed so that he could no longer sit, stand or feed himself. Intravenous lorazepam helped but his epileptic spasms and atypical absences became more intrusive. He was started on prednisolone, following which there was an improvement in his behaviour, functioning and seizures.

Individual 4 had further admissions over the next few months. He was having multiple GTCS per day. His EEG at 2 years 7 months showed a modified hypsarrhythmia (high amplitude delta activity with frequent sharp and slow wave complexes, consistent with an epileptic encephalopathy). He was started on pyridoxine and pyridoxal phosphate without improvement. Seizures recurred following weaning of prednisolone with facial twitching, hypersalivation, epileptic spasms, and tonic seizures. A gastrostomy was placed due to poor feeding.

At 3 years and 2 months of age he was admitted again with suspected NCSE. Medication changes improved his clinical state but he has been admitted occasionally (once or twice a year) since then diagnosed either clinically or on EEG to have NCSE. A medium-chain triglyceride (MCT)-based ketogenic diet was introduced at 3 years and 10 months of age. There was marked improvement in alertness over the next 6 weeks. However, he was worse when reviewed at 4 years and 1 month of age. The ketogenic diet was changed to a classical diet at 4 years and 5 months of age and weaned off at 4 years and 10 months of age. He was reluctant to walk, suck or swallow during this period. He experienced recurrent urinary tract infections despite prophylactic

trimethoprim. DMSA indicated that one kidney had 30% of normal function. He underwent unilateral nephrectomy at 4 years of age.

At 10½ years of age, individual 4 had worsening seizures. He had been on nitrazepam and this was converted to clobazam. He was admitted for frequent tonic seizures and was given intravenous phenobarbitone. Maintenance phenobarbitone was started at 10 years and 10 months of age. He had a relatively stable period of several months on a combination of valproate, clobazam and lacosamide. He was still having several seizures per week but was generally happy and alert.

At 11 years and 4 months of age he deteriorated and was re-admitted to hospital. He was having up to 300 atypical absence seizures per day. EEG revealed almost continuous generalised spike and slow wave activity. Ambulatory EEG was consistent with Lennox-Gastaut syndrome. He was having a mixture of atypical absences, tonic spasms and some tonic seizures. There was an attempt to wean him off nitrazepam, lacosamide and, subsequently, phenobarbitone. However, his seizures increased (occurring every few minutes), and he required HDU admission for intravenous midazolam. He was gradually weaned onto oral diazepam which proved useful. Ethosuximide and cannabidiol were started. Following weaning of the diazepam he again had frequent absence seizures and increased tonic seizures (up to 20+ per day). These improved with intravenous phenobarbitone and lorazepam. His functioning reduced markedly, and he became very subdued. He had prolonged periods of inactivity and looking glazed. This was unusual for individual 4 who was usually very active. Repeat EEG showed NCSE. Clobazam was recommenced leading to significant improvement and he was discharged after 2 months in hospital. There were concerns about retching and vomiting towards the end of this inpatient stay. He was fed partly orally and partly by gastrostomy. He was treated with ondansetron, omeprazole and domperidone A pH study showing slight reflux to the upper oesophagus

At 11 years and 8 months of age individual 4 was admitted again for several weeks. Initially, he was having up to 70 tonic seizures overnight which improved following an increase in clobazam. Due to concerns about self-mutilating behaviour, and subsequent raised γ-glutamyl transferase

(GGT) and alanine aminotransferase (ALT) he was slowly weaned off clobazam. He continued to be on sodium valproate although this was reduced slightly prior this prolonged admission.

Individual 4 was last reviewed at 12 years of age. He had severe intellectual disability and an autism spectrum disorder. He continued to have prolonged admissions for recurrent episodes of NCSE associated with frequent vomiting. He lost motor skills during periods of frequent seizures. When he was well, he could walk independently, though he required supervision and often one-hand support. He would have eye contact and smile responsively as well as vocalise. He did not have recognisable speech. He often ground his teeth. He had not experienced early puberty. Individual 4 was suspected to have cortical visual impairment. He walked with a broad-based unsteady gait. On examination he had eczema, hypermobile joints and mild hirsutism. Neurologically he had low truncal tone, mild scoliosis, tight hamstrings but no lateralising signs. His weight was 39 kg (+0.1 SD) and OFC 53 cm (-1.3 SD).

Individual 4's genetic tests have included aCGH, and single gene testing for *SLC2A1* and *STXBP1*. Other investigations with normal results have included CSF neurotransmitters and pyridoxal phosphate, muscle biopsy (histopathology and respiratory chain enzymes), urine AASA and transferrin isoelectric focusing. Brain MRI scan at 1½ years of age was normal. Brain MRI at 12 years of age showed slight cerebellar volume loss and decreased volume of the splenium of the corpus callosum. There was a small arachnoid cyst in the left middle cranial fossa. There was possible increased signal and swelling of the hippocampi bilaterally which may have reflected seizure activity. Individual 4 had a 3T MRI brain scan at 13 years of age which showed symmetrical T2 hyperintensity of the hippocampal body and head with loss of definition of the internal architecture on all the sequences. There was no evidence of underlying diffusion restriction. Review of a previous MRI scan from 2½ years of age showed similar changes.

Summary of antiepileptic medications (current anticonvulsant drugs underlined): <u>sodium valproate</u>, levetiracetam, ethosuximide (caused vomiting), topiramate, lacosamide, rufinamide (caused vomiting), lamotrigine (associated with nonconvulsive status), phenytoin (not used since 2½ years of age due to concerns that it resulted in NCSE when maintenance dose was given), phenobarbital (some benefit though not significant recently with tonic or absence seizures),

clobazam (useful for NCSE), nitrazepam (longstanding use), prednisolone (none since 2 years of age), lorazepam (used for NCSE and improved tonic seizures), diazepam, cannabidiol (Epidyolex), zonisamide.

Individual 5

This male individual is the first child of unrelated White Canadian parents. He was born by spontaneous vaginal delivery following an uncomplicated pregnancy at 39+3 weeks gestation. His biometry at birth was weight 3.67 kg (+0.7 SD), OFC 36.5 cm (+1.5 SD) and length 53.5 cm (+1.7 SD). He had mild hyperbilirubinemia which did not require treatment. There was no arthrogryposis or talipes. He began having seizures at 5 days of age. These involved repetitive right eye blinking, gaze and head deviation, repetitive swallowing and clicking noises. Seizures lasted 1 to 2 minutes and occurred 1 to 5 times a day. MRI brain at 1 month of age found no structural abnormalities and no evidence of acute ischemia. EEG was abnormal and showed epileptiform discharges from the right temporal region and independent epileptiform discharges from the left temporal region. This suggests multiple epileptogenic foci. Some of the events were associated with leg twitching.

Individual 5 smiled social at 8 weeks of age but underwent a period of regression around 4 months of age. Individual 5 was last reviewed at 2 years and 3 months of age. He had severe developmental delay. He was not talking, sitting or standing independently. He would occasionally (but not consistently) fix and follow light. Individual 5 was gastrostomy fed due to problems with suck and swallow coordination. He had constipation, cortical visual impairment, moderate conductive hearing loss, hypothyroidism, osteopenia, and gastroesophageal reflux disease. He continued to have focal dyscognitive seizures. Individual 5 had periodic abnormal posturing (decorticate and opisthotonus) as well as dystonic episodes and myoclonus. On examination, individual 5 was not dysmorphic. Growth parameters (at 22 months of age) were weight 15.8 kg (+2.4 SD) and OFC 48.5 cm (-1.1 SD). Neurological examination found generalised hypotonia with no contractures or scoliosis. His knee reflexes were normal with no clonus. He had exotropia with normal fundi.

Individual 5's most recent EEG was done at 2 years of age. This showed almost continuous 1.5-2 Hz sharp and slow wave complexes during sleep and also appearing frequently during wakefulness. The electroclinical features were suggestive of Lennox-Gastaut syndrome. Individual 5's genetic testing has included aCGH and epilepsy gene panel (Courtagen epiSEEK) which were both normal. Extensive metabolic investigation including lactate, plasma amino acids, urine organic acids and urine metabolic screen were all negative.

Summary of antiepileptic medications:
Phenobarbital, valproic acid (individual 5 experienced abdominal pain with valproic acid), clobazam, rufinamide, levetiracetam, ACTH, cannabis, vigabatrin, lamotrigine, topiramate, and the ketogenic diet. Nothing has worked well to date - new medications and doses are still being tried.

Individual 6

This male individual (Decipher ID 264848) is the child of unrelated white British parents. He has a brother with infantile-onset epilepsy, developmental delay and behavioural problems, and a maternal aunt diagnosed with cerebral palsy and epilepsy. Further clinical evaluation or genetic testing of individual 6's brother or parents has not been possible.

The pregnancy with the individual 6 was uncomplicated. Scans were normal during pregnancy and there was no bleeding. He was born by spontaneous vaginal delivery at 38 weeks gestation. His birth weight was 2.6 kg (-1.2 SD). He was admitted to the neonatal unit within 24 hours following a cyanotic episode. Investigations showed gastroesophageal reflux and he was treated with Gaviscon. He was discharged home after 3 weeks. His mother continued to be concerned during infancy about the number of cyanotic episodes individual 6 was having. After several months he was admitted to hospital. In retrospect, it was realised the cyanotic episodes may have been seizures. In addition, he was found to have an atrial septal defect.

Individual 6 went on to be diagnosed with global developmental delay, seizures, and autism. He sat at 1 year and walked at 2 years and 3 months. Individual 6 was last assessed at 5 years 8

months. His OFC was 49 cm (-2.6 SD). His examination findings included strabismus and coarse facial features. Genetic investigations of individual 6 have included aCGH and testing for Fragile X syndrome. The results were normal.

Individual 7

This male individual is the child of consanguineous Chinese parents (half-first cousins). He had a family history of epilepsy in a paternal grand uncle. He was delivered by Caesarean section at 35+2 weeks gestation following an uncomplicated pregnancy. His birth weight was 2.5 kg (+0.1 SD). There were no concerns in the neonatal period. Individual 7 smiled socially at 3 months, sat unsupported at 9 months and walked independently at 13 months. The first concerns were at 6 months of age when he developed focal seizures.

Individual 7 experienced a period of regression at 14 months of age. He lost the ability to walk, sit, or smile socially. EEG at 1 year and 11 months of age showed generalized spike and slow-spike waves. There was atypical hypsarrhythmia and intermittent burst suppression during sleep. Clusters and isolated spasms were detected when he was awake. At 3 years and 2 months his head circumference was 48cm (-2.6 SD).

Individual 7 was last reviewed at 5 years and 8 months old. He had severe developmental delay and intractable epilepsy. He was not yet talking, walking or toilet trained. He was fed orally with pureed food, but choking was a frequent problem. He had problems with sleep disturbance. His predominant seizure type are focal seizures. He has also had myoclonic seizures, epileptic spasms and GTCS. On examination his length was 110 cm (-0.8 SD) and weight 18kg (-0.9 SD). Neurological examination found limb hypertonia with positive Babinski sign and ankle clonus. There was no strabismus, joint contractures or abnormal movements.

Individual 7's genetic testing has included aCGH. The results were normal. Metabolic investigations were also negative. Brain MRI at 3 years and 2 months of age showed cerebral cortex atrophy with bilaterally enlarged ventricles.

Summary of antiepileptic medications (current anticonvulsant drugs underlined): Levetiracetam, valproate, clonazepam, carbamazepine (made symptoms worse), zonisamide (worked well), oxcarbazepine, lamotrigine (allergic reaction), ACTH (worked well).

Genomics England Research Consortium

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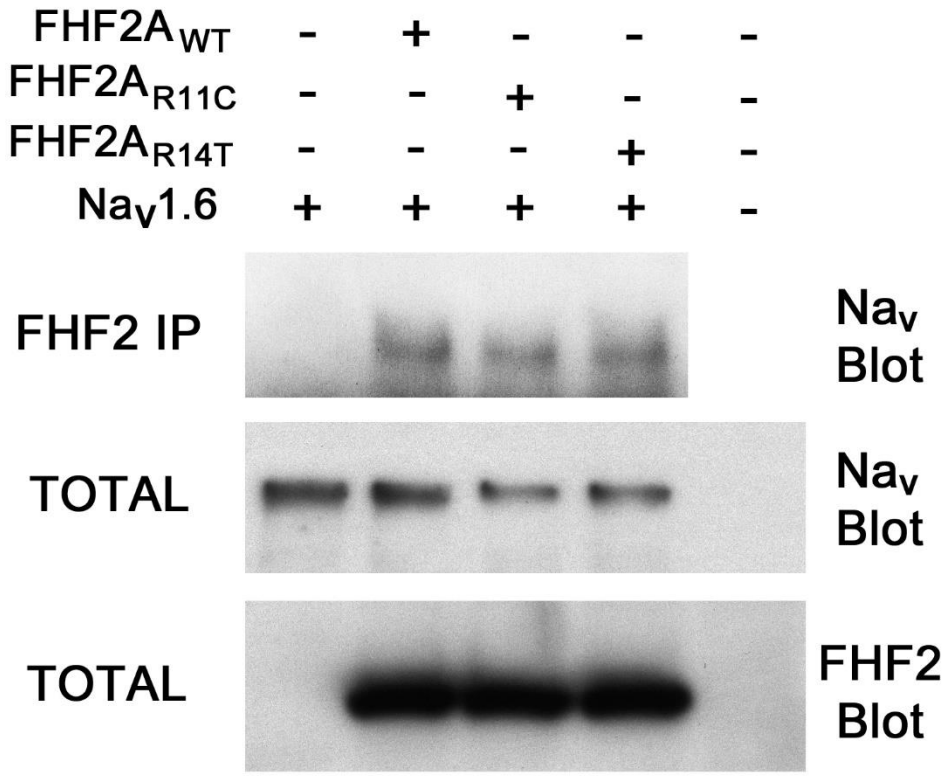

Figure S1. FHF2A$_{WT}$, FHF2A$_{R11C}$ and FHF2A$_{R14T}$ proteins interact with Na$_v$1.6. Neuro2A cells were transiently transfected with expression vectors indicated at top of figure. Total lysates or anti-FHF2 immunoprecipitates (IP) were subjected to gel electrophoresis and immunoblotting with anti-FHF2 or anti-pan-Na$_v$ antibodies. Na$_v$1.6 is detected in anti-FHF2 immunoprecipitates from cells expressing FHF2A$_{WT}$, FHF2A$_{R11C}$ or FHF2A$_{R14T}$.

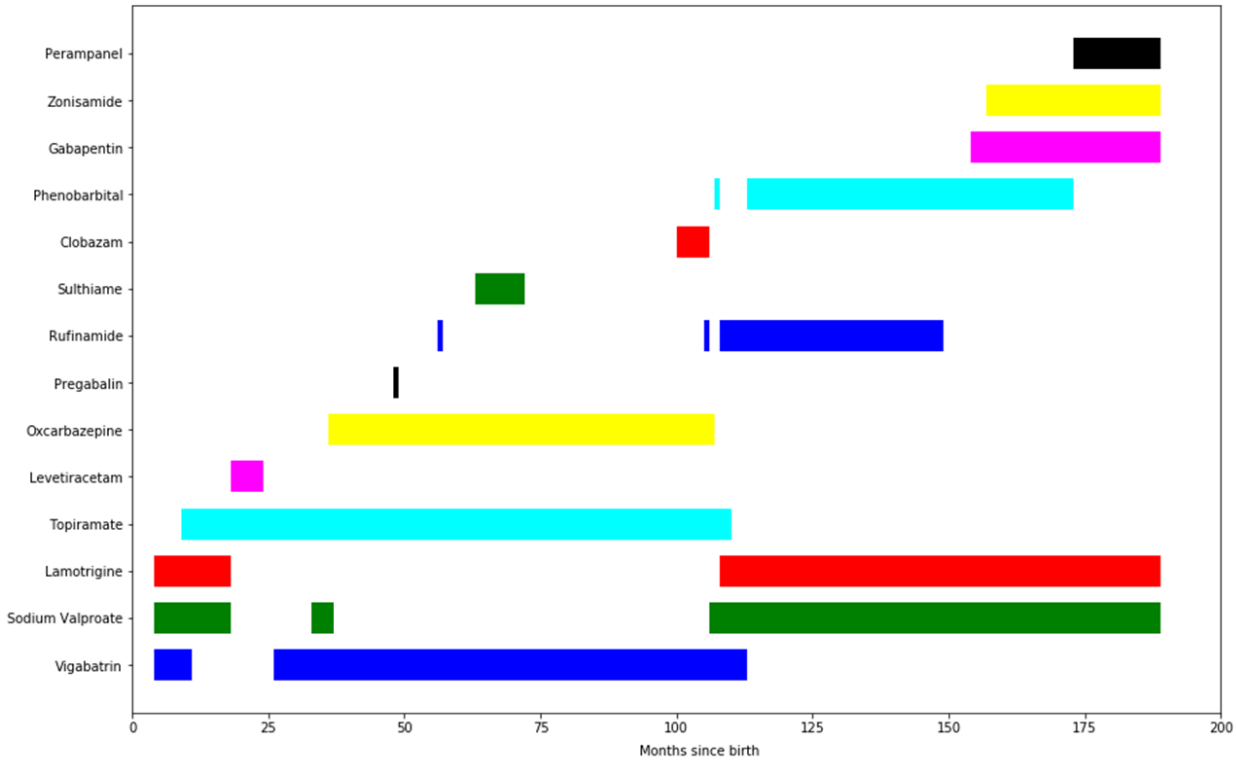

Figure S2. Antiepileptic medications given to individual 1. Timeline illustrating the combinations of anticonvulsant drugs (vertical axis) used to treat individual 1 from birth to last review.

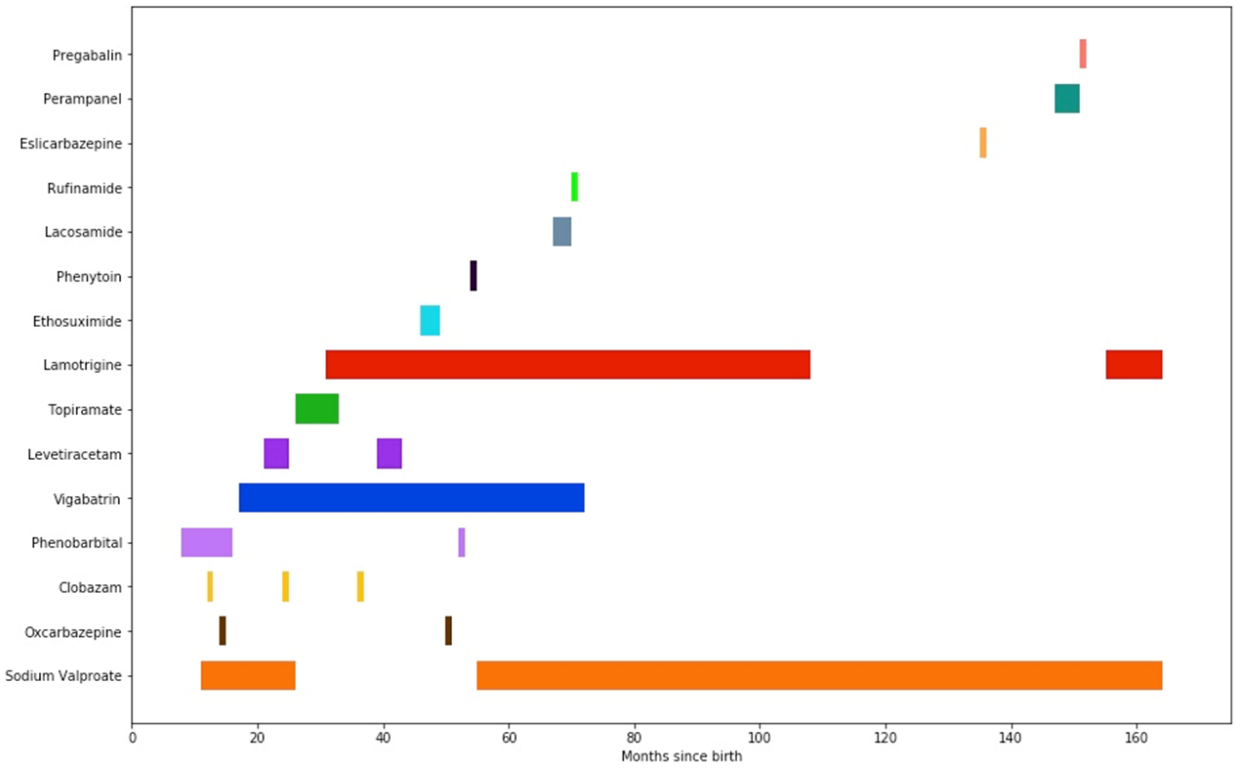

Figure S3. Antiepileptic medications given to individual 2. Timeline illustrating the combinations of anticonvulsant drugs (vertical axis) used to treat individual 2 from birth to last review.

Variant	*FHF2*, c.31C>T, p.(Arg11Cys)		*FHF2*, c.41G>C, p.(Arg14Thr)		*FHF2*, c.32G>C, p.(Arg11Pro)	
Algorithm	Score	Prediction	Score	Prediction	Score	Prediction
SIFT	0.001	Damaging	0	Damaging	0.057	Tolerable
Polyphen-2 (HDIV)	1	Probably damaging	0.875	Possibly damaging	0.276	Benign
Polyphen-2 (HVAR)	0.983	Probably damaging	0.58	Possibly damaging	0.212	Benign
LRT	-	-	-	-	-	-
MutationTaster	1	Disease causing	1	Disease causing	1	Disease causing
MutationAssessor	1.935	Low	1.935	Low	1.935	Low
FATHMM	-1.94	Damaging	-1.92	Damaging	-1.93	Damaging
PROVEAN	-2.27	Tolerable	-1.99	Tolerable	-1.96	Tolerable
VEST3	0.791	Damaging	0.702	Damaging	0.872	Damaging
MetaSVM	0.383	Damaging	0.305	Damaging	0.087	Damaging
MetaLR	0.666	Damaging	0.594	Damaging	0.548	Damaging
M-CAP	0.473	Damaging	0.388	Damaging	0.322	Damaging
CADD	22.4	Damaging	22.9	Damaging	22.8	Damaging
DANN	0.999	Damaging	0.973	Tolerable	0.994	Damaging
FATHMM_MKL	0.893	Damaging	0.97	Damaging	0.97	Damaging
Eigen	-	-	-	-	-	-
GenoCanyon	1	Damaging	1	Damaging	1	Damaging
fitCons	-	-	-	-	-	-
GERP++	3.36	Conserved	4.29	Conserved	4.29	Conserved
phyloP	2.668	Conserved	7.364	Conserved	7.364	Conserved
phastCons	1	Conserved	1	Conserved	1	Conserved
SiPhy	10.765	Nonconserved	15.342	Conserved	15.342	Conserved
REVEL	0.697	Damaging	0.733	Damaging	0.687	Damaging
ReVe	0.889	Damaging	0.863	Damaging	0.918	Damaging
ClinPred	0.86299818	Pathogenic	0.90703922	Pathogenic	0.812778	Pathogenic

Table S1. Results of *in silico* prediction tools for the three *FHF2* (NM 004114.5) missense variants.

Supplemental Material and Methods

Electrophysiology and immunochemical analyses of plasmid-transfected Neuro2A cells

All procedures parallel those we have described previously.[1,2] The $Na_v1.6$ expression plasmid (pIRESneo3-$Na_v1.6^{TTXr}$) bears the tetrodotoxin (TTX) binding site substitution Y371S, rendering the channel resistant to TTX. FHF2A/GFP bicistronic expression plasmid pIRES2-FHF2A-ZsGreen drives coexpression of untagged FHF2A and green fluorescent protein, while pIRES2-ZsGreen expresses fluorescent protein only. Missense substitutions p.R11C or p.R14T were introduced into the pIRES2-FHF2A-ZsGreen plasmid using complementary mutagenic primers and PfuTurbo DNA Polymerase (Agilent).

Neuro2A cells were used for expression of sodium channels and FHFs by Lipofectamine (LFN2000)-mediated plasmid transfection at a 2:1 ratio of Na_v- and FHF-expressing plasmids. For protein expression analysis, transfected cells were lysed after 24 hour culture. For electrophysiology, transfected cells were trypsinized, plated onto coverslips, and maintained for 24–48 hours before transfer to the recording chamber.

For analysis of $Nav1.6^{TTXr}$-mediated inward sodium currents, O_2:CO_2 (95:5; carbogen)-bubbled extracellular solution contained the following (in mM): 109 NaCl, 26 $NaHCO_3$, 10 HEPES, 4.7 KCl, 11 glucose, 1.2 $MgCl_2$, 2 $CaCl_2$, 0.2 $CdCl_2$, 3 myo-inositol, 2 Na pyruvate, 0.001 TTX (buffered to pH 7.2 with NaOH), and the intracellular pipette solution (Pipette Solution I) contained 104 CsF, 50 tetraethylamine chloride (TEA), 10 HEPES, 5 glucose, 2 $MgCl_2$, 10 EGTA, and 2 Na_2ATP, 0.2 Mg-GTP (buffered to pH 7.2 with CsOH). The recording chamber was set within a Nikon EF600 microscope equipped with 40X water-immersion objective and video camera. Transfected cells were identified by green fluorescence and whole-cell configuration was induced after achieving a 5–20 Gohm seal. Wide pipette tips were used yielding a series resistance of 2.5–5 Mohm following whole cell access. Voltage-clamp was conducted using an Axopatch 200B amplifier, Digidata 1322 digital/analog interface, and pCLAMP9 software (Molecular Devices). Recording signals were filtered at 5 kHz and digitized at 20 kHz. For measurements of evoked sodium currents, capacitive and leak currents were

subtracted during data acquisition using the presweep hyperpolarizing P/N method in the pCLAMP9 software.

Protocols for measuring voltage dependence of sodium channel steady-state inactivation and accumulation into and recovery from long-term inactivation were described previously.[1] To assay steady state inactivation, cells were depolarized for each sweep from -110 mV holding potential to test potentials between -110 mV to -20 mV in 5 mV intervals for 80 ms after which sodium current from non-inactivated channels was induced by further depolarization to -10 mV. To assay accumulating long-term inactivation, cells were subjected to four consecutive depolarizations from -90 mV to 0 mV for 16 ms separated by 40 ms -90 mV recovery phases. Progressively decreasing sodium current in the second, third, and fourth depolarizations reflects long-term channel inactivation. To assay for recovery rate from long-term inactivation, cells were subjected to three successive depolarizations from -90 mV to 0 mV to accumulate long-term inactivation, and a fourth depolarization was given at time intervals ranging from 20 ms to 1 s. Recovery from intrinsic fast inactivation occurs within 20 ms, so fraction recovery from long-term inactivation at time t was calculated as $\{I_{Na}(t) - I_{Na}(20)\}/\{I_{Na}(0) - I_{Na}(20)\}$, where $I_{Na}(0)$ is the sodium current generated from the first depolarization, $I_{Na}(20)$ is the sodium current from the fourth depolarization following 20 ms recovery, and $I_{Na}(t)$ is the sodium current from the fourth depolarization following recovery for time t. All data points in the graphs are expressed as mean +/- standard error of values from all (n) recorded cells. Statistical significance of differences in data point values between pairs of transfected cells was assessed by Student T test.

For biochemical analyses, cells were lysed in buffer containing (in mM) 137 NaCl, 20 Tris pH 7.4, 2 EDTA, 25 β-glycerophosphate, 2 $Na_4P_2O_7$, 1 Na_3VO_4, 10% glycerol, 1% Triton X-100 and then clarified by high-speed centrifugation. Lysates were electrophoresed through 4%-20% polyacrylamide SDS gels directly or after immunoprecipitation with rabbit polyclonal antibodies against the C-terminal domain of FHF2.[1] Proteins were electrophoretically transferred from gel to polyvinylidene difluoride (PVDF) membrane and probed with either the same FHF2 antibodies or with pan-sodium channel monoclonal antibody K58/35 (Sigma Aldrich) followed

by incubation with secondary peroxidase conjugated antibodies and enhanced chemical luminescence detection

X-chromosome inactivation analysis

Two assays comprising amplification of a short fragment of X chromosome (amplimer) were used to determine the activation status of each X chromosome. Each amplimer contains a polymorphic marker adjacent to the promoter of a gene which is unmethylated on the active X chromosome and methylated on the inactive chromosome. The two genes investigated were the androgen receptor *AR* and *ZNF261* (ZMYM3; DXS6673E). Both genes are in Xq13. Prior to amplification, both assays employ methylation-sensitive restriction enzymes, HpaII and CfoI, which cut at the promoter site on the active (unmethylated) but not on the inactive (methylated) X chromosome, using previously described methods.[3,4] To calculate the X-inactivation ratio, each sample was set up in duplicate: one digest and one mock digest without enzyme. Both were then amplified by PCR using a fluorescently labelled primer. The ratios of the heights of the two peaks were then compared between the undigested and digested samples. To evaluate the significance of patterns of X inactivation the ratios are compared with data from a sample of >1,000 phenotypically unaffected females.[5]

References for Supplemental Material and Methods

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