The American Journal of Human Genetics, Volume *101*

Supplemental Data

De Novo Mutations in *YWHAG* Cause Early-Onset Epilepsy

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Supplemental Note: Case Reports

Subject-A (SCL1A2; c.244G>A, p.Gly82Arg)

A 6.5 year old boy with developmental and epileptic encephalopathy, profound global delay, early developmental arrest, cortical visual impairment, feeding difficulties and a hyperkinetic movement disorder. The pregnancy and delivery were uncomplicated. His parents were non-consanguinous, and had no family history of neurological disorders.

Seizure disorder and treatment

He presented at 6 weeks of age with prolonged focal motor seizures and reduced visual attentiveness. He initially developed left clonic leg jerks during wakefulness and sleep. This evolved to right or left limb involvement with impaired awareness or bilateral tonic-clonic seizures. His EEG at 2 months showed paroxysmal delta and frequent independent bilateral mid-central parietal spikes and sharp waves (right > left) and occasional right temporal spikes. His seizures initially responded partially to phenobarbital and levetiracetam, but he had seizures every 1 to 6 months. The longest seizure free period was 16 months, between 4 and 6 years. Seizures were often prolonged and/or occurred in cluster. Currently, he has been seizure free 5 months on phenobarbital, levetiracetam and clobazam. He did not tolerate topiramate. His EEG at 4 years revealed a slow dysrhythmic background with multifocal epileptiform discharges.

Development

Subject-A development never progressed past the infantile level; however, there was no definite regression until 4 years 9 months when he was hospitalized for lethargy, irritability and focal motor status epilepticus. Following seizure control, the lethargy and irritability improved, but he could no longer tolerate oral feeds and a G-tube was placed at 6 years. At 6.5 years, he is non-verbal, and unable to sit unsupported. His head circumference remains at the 50^{th} percentile. He has reduced visual attentiveness, sluggishly reactive pupils, axial hypotonia, mild limb spasticity and dyskinetic movements of his arms.

Neuroimaging

MRI of the brain at 1 month was normal. At 2 years, there was progressive atrophy, a thin corpus callosum, delayed myelination and abnormal white matter signal in the peritrigonal region. At 4 years there was further progressive generalized atrophy; abnormal signal in the basal ganglia and thalami; and periventricular white matter signal abnormalities (see Figure 2).

Other testing

A muscle biopsy at 5 years showed non-specific changes, including increased fibre size variability and type II fibre atrophy. Muscle mitochondrial respiratory enzymes were normal.

Subject-B (YWHAG; c.394C>T, p.Arg132Cys)

An 18 year old woman with developmental and epileptic encephalopathy, mild to moderate intellectual disability, attention deficient hyperactivity disorder (ADHD), anxiety, obsessive compulsive traits and an action tremor.

Seizure disorder and treatment

Seizures were first noted at 12 months but were undiagnosed until 27.5 months. Seizures were characterized by staring with eyelid fluttering with or without limb jerks. Video-EEG examination showed dysrhythmic background and frequent generalized atypical spike and wave, fragmentary spike and wave and frequent independent bilateral frontal spikes. She had multifocal myoclonic jerks some of which correlated with generalized spike and wave. Her seizures responded well to clonazepam, with only rare myoclonic jerks during sleep. However, clonazepam was discontinued at 8 years, and her EEG showed occasional fragmentary generalized spike and wave off medication. However, myoclonic seizures recurred and she also developed atypical absence and rare generalized tonic-clonic seizures. Lamotrigine proved ineffective. Seizures were stopped on Divalproex sodium and ethosuximide and she has been seizure free since 11 years. She has been on Divalproex sodium monotherapy since age 17 years. Her most recent EEG at 14 years revealed a dysrhythmic background and rare sharp waves in the bilateral anterior quadrants.

Development

Early development was normal except for language delay noted at 2 years, but with time, global developmental delay became more apparent. Neuropsychological assessment at 11 years confirmed mild to moderate intellectual disability and ADHD. Her ADHD symptoms improved on methylphenidate. She also has anxiety with obsessive compulsive traits, migraine and sleep difficulties. The maternal family history is significant for migraine and depression. Physical examination revealed normal head circumference, action tremor with mild coordination difficulties.

Neuroimaging

MRI of the brain performed at 3 years revealed an asymmetric brainstem of unlikely clinical significance.

Subject-C (SCL1A2; c.866C>G, p.P289R)

A 10-year old boy who presented with staring spells, who is the only child to healthy Caucasian parents who are first cousins. He has myoclonic jerks, absence and gelastic seizures and severe global developmental delay.

Seizure disorder and treatment

Subject-C developed twitching of his right foot in the first week of life followed by intermittent head turning to the right and twitching of his right arm in the third week and eye blinking with stiffening of the extremities at 4 weeks of age. From 7 weeks he had constant twitching of his legs..He was initially treated with phenobarbital which resulted in a seizure-free interval of 2 weeks. He then developed clusters of seizures which did not respond to phenytoin, rectal paraldehyde, midazolam, pyridoxine and clonazepam. An EEG at 3 months showed bilateral frontal sharp and slow wave discharges associated with arrest of activity and staring. Three weeks later he developed perioral twitches, myoclonic jerks involving the hands, feet and shoulders. His EEG showed irregular high-amplitude slow activity (delta) focal on the right and focal sharp waves in the left temporal area. Various combinations of anticonvulsants were tried with little response. There was a reduction in the frequency of his myoclonic jerks with some improvement in his development following a course of prednisolone, but there was no change in his EEG. At 6 years he continued to have 50 seizures daily on Rufinamide, Levetiracetam, Nitrazepam and the ketogenic diet.

Development

At 4 months, he had treatment resistant myoclonic seizures with loss of skills. He was unable to fix or follow. His head circumference was on the 91st percentile. There were no dysmorphic features nor neurocutaneous findings. There was hypotonia, with no head control, weak Moro and grasp reflexes, albeit with normal power and deep tendon reflexes in all limbs. He had a gastrostomy tube and fundoplication at 3 years. He required bilateral femoral osteotomies at 5 years. He has a tracheostomy and also developed severe scoliosis.

Neuroimaging

An initial MRI of the brain at 2 months was normal but at 2.5 years a second scan showed severely delayed myelination, non-progressive cerebral atrophy, and T2 prolongation in caudate heads and putamina bilaterally.

Other testing

Neurometabolic testing including analysis of CSF was normal. Standard karyotype and methylation analysis for Angelman syndrome were normal. Eye examination showed healthy maculae. No mutations

were identified in *SCN1A*, *MECP2*, *POLG*, *PEO1* or *ARX* genes. There was no evidence of the common mitochondrial DNA mutations causing MELAS (m.3243A>G) and MERRF (m.8344A>G) in lymphocyte DNA. He was entered into the DDD Study for trio whole exome sequencing. WES identified a *de novo* missense variant in the *SLC1A2* gene (c.866C>G; p.Pro289Arg) and he was compound heterozygous for two missense variant in the *SLX4* gene (c.1372A>G; p.Lys458Glu, maternally inherited, and c.2305G>C; p.Glu769Gln paternally inherited). These results were validated by Sanger sequencing in the proband and his parents. The *SLC1A2* variant was classified as likely pathogenic whereas the *SLX4* variants were classified as being of uncertain significance. Biallelic mutations in *SLX4* cause Fanconi anaemia Type P, but his full blood count showed no evidence of pancytopenia and his Mitomycin C test for Fanconi anaemia was normal. Chromosome microarray was normal.

Subject-D (YWHAG; c.44A>C, p.E15A)

A 10-year-old girl who is the only child of non-consanguineous parents. She has three paternal half-siblings. Pregnancy and delivery were uncomplicated. However, her mother has macrocephaly and reported a single febrile seizure.

Seizure disorder and treatment

Subject-D had two prolonged febrile seizures, at 6 and 12 months of age. After her first seizure, she showed developmental delay and hypotonia. At 2 years she had *status epilepticus* lasting about 6.5 hours, after which she developed a hemiparesis and regression of psychomotor development. At age 3, she had a second episode of *status epilepticus* followed by (reversible) cognitive deterioration. Currently, her epilepsy is relatively well controlled on divalproex sodium.

Development

She walked at 4 years but with splints because of her low muscular tone. She started to speak at 4.5 years. Her development is progressing slowly; her IQ is 55. On physical examination, her head circumference was normal (+1 SD), she had a broad nasal bridge with telecanthus, joint hyperlaxity, and a few café-au-lait spots at her back. *Neuroimaging*

MRI of the brain showed generalized atrophy.

Other testing

Ophthalmologic examination was normal with no Lisch nodules. Exome sequencing also identified a *de novo* variant in the NF1 gene of unknown significance.

Subject-E (YWHAG; c.394C>T, p.Arg132Cys)

A 22 year old female with a diagnosis of generalized epilepsy, the second child to healthy, unrelated Caucasian parents.

Seizure disorder and treatment

Subject-E parents' originally sought medical advice because they had witnessed jerks, predominantly of the upper limbs, in the first six months of life and on a few occasions they noticed their daughter's eyes rolling. At 18 months, she had a generalized tonic-clonic seizure which lasted about 45 minutes, associated with fever. Diazepam was required to terminate the seizure. At 20 months, she developed myoclonic jerks which occurred 30-40 times per week. An EEG showed bursts of generalized 3Hz spike and slow wave discharges associated with clinical absence seizures. Subject-E was started on sodium valproate and had a seizure free period of a month. Subsequently, she had upper limb jerks with absences, and had a further generalized tonic clonic seizure the following year. Various drug regimens were tried, all including sodium valproate, but without great effect. At 10 years, she was having up to 100 myoclonic seizures per day and video-telemetry at that time showed frequent bursts of very large amplitude multiple sharp, polyspike and slow wave activity lasting 1-6 seconds. During sleep there was almost continuous multiple spike and slow wave complexes at 1-3Hz. At 14 years, a trial of stiripentol was initiated in conjunction with sodium valproate and topiramate and there was a marked improvement in her seizure control. She had one normal EEG six months after starting stiripentol but a subsequent EEG showed occasional spike and wave activity. Currently she has occasional absences usually lasting a couple of seconds and occasional myoclonic jerks which tend to occur in clusters. Topiramate has been discontinued.

Development

In early life there were some minor feeding difficulties, and her tongue protruded. On examination, she was not dysmorphic. Nevertheless, her head circumference was on the 20^{th} percentile. It became evident by 8 months that she was not achieving her developmental milestones, as she was unable to sit unsupported. However, she did make slow developmental progress and walked at 16 months. She had a wide-based gait, poor coordination and ataxia. While there was evidence of motor developmental delay prior to the onset of seizures, her progress slowed at the onset of seizures. She attended a special school for children with moderate learning difficulties and she now attends a specialist boarding college for individuals with complex epilepsy. She has relatively good expressive language and is able to read words but her numeracy is poor. She has a recent diagnosis of autism spectrum disorder. At 16 years she was noted to have scoliosis that progressed rapidly to 45^{o} over a period of months.

Neuroimaging

Her brain MRI was normal at 10 years.

Other testing

Karyotype and Comparative Genomic Hybridization (CGH) array were normal, and no pathogenic variants were identified in *SCN1A* or *PCDH19*. She was entered into the DDD study for trio whole exome sequencing and a *de novo* missense heterozygous variant in *YWHAG* was identified.

Subject-F (YWHAG; c.394C>T, p.Arg132Cys)

This 15-year-old girl is the only child of non-consanguineous French parents. Pregnancy and delivery were uncomplicated. She has one paternal elder half-brother. A maternal aunt has epilepsy and there is no family history of other neurological problems.

Seizure disorder and treatment

At 6 years, she had multiple generalized seizure types: absence, eyelid myoclonia, myoclonic and probable myoclonic-atonic seizures. Her parents were unable to recall the age at seizure onset. Her seizures are relatively well controlled on divalproex sodium and lamotrigine (except for occasional absence seizures).

Development

At 2 years, she was referred for assessment of motor and speech (she walked at 19 months). She had mild dysmorphic features. At 6 years IQ was evaluated (Verbal IQ 73, Performance IQ 58). On physical examination at 15 years, she has a normal head circumference (+0 SD), facial dysmorphism and thoraco-lumbar kyphoscoliosis.

Neuroimaging

Brain MRI was normal.

Table S1: list of analyzed genes, prioritized based on brain-specific co-expression with established EE genes

GENE	RefSeq	Genomic coordinates (hg19)	Coverage 10x (%)
ACOT4[a]	NM_152331	chr14:74058663-74062358	84%
AKAP6	NM_004274	chr14:32902699-33293979	100%
ANK3	NM_001204403	chr10:61802448-62493077	100%
CRTAC1	NM_018058	chr10:99625304-99790229	99%
DAO	NM_001917	chr12:109278782-109294311	100%
FBXO41[a]	NM_001080410	chr2:73486109-73496758	83%
GABRB1	NM_000812	chr4:47033668-47428035	100%
GABBR2[a]*	NM_005458	chr9:101052865-101471019	86%
IQSEC2	NM_001111125	chrX:53263400-53350321	92%
KNDC1[a]	NM_152643	chr10:134973971-135038394	86%
MAST1	NM_014975	chr19:12949386-12985684	94%
NBEA[a]	NM_015678	chr13:35516957-36245128	97%
PACS2	NM_001100913	chr14:105781255-105861009	96%
PAK6	NM_001128628	chr15:40556986-40568295	100%
PLXNA1	NM_032242	chr3:126707436-126752860	100%
RALGPS1	NM_014636	chr9:129724568-129981048	100%
SELRC1[a]	NM_023077	chr1:53153391-53163998	100%
SLC1A2	NM_004171	chr11:35282440-35440513	100%
TRIO	NM_007118	chr5:14143834-14508531	94%
YWHAG	NM_012479	chr7:75958893-75988125	100%

[a] denotes 6 additional genes prioritized by Oliver et al. [13].