

## Supplemental information

### **A reverse genetics and genomics approach to gene paralog function and disease: Myokymia and the juxtapanode**

**Dana Marafi, Nina Kozar, Ruizhi Duan, Stephen Bradley, Kenji Yokochi, Fuad Al Mutairi, Nebal Waill Saadi, Sandra Whalen, Theresa Brunet, Urania Kotzaeridou, Daniela Choukair, Boris Keren, Caroline Nava, Mitsuhiro Kato, Hiroshi Arai, Tawfiq Froukh, Eissa Ali Faqeih, Ali M. AlAsmari, Mohammed M. Saleh, Filippo Pinto e Vairo, Pavel N. Pichurin, Eric W. Klee, Christopher T. Schmitz, Christopher M. Grochowski, Tadahiro Mitani, Isabella Herman, Daniel G. Calame, Jawid M. Fatih, Haowei Du, Zeynep Coban-Akdemir, Davut Pehlivan, Shalini N. Jhangiani, Richard A. Gibbs, Satoko Miyatake, Naomichi Matsumoto, Laura J. Wagstaff, Jennifer E. Posey, James R. Lupski, Dies Meijer, and Matias Wagner**

# SUPPLEMENTAL NOTE: CASE REPORTS

## FAMILY 1

### Family 1, II-3

The index subject of Family 1 is the second child of consanguineous parents from Pakistan.

His older sister had severe growth delay and was consequently diagnosed with growth hormone (GH) deficiency due to a pathogenic homozygous variant in *GHRHR*. She is successfully treated with GH. In the index subject, global developmental delay as well as growth delay was noted early on, but the boy never had developmental regression. He had muscular hypotonia and poor feeding but never required tube feeding. The same pathogenic variant in *GHRHR* was identified explaining growth delay and GH treatment was initiated at the age of 7 months. He was orthopedically treated for knee contractures for several years. He is currently 8 years old, and his size is 119.2 cm (<1 centile, SDS -2.91), his weight 19.75 kg (<1 centile, SDS -3.07) and his OFC is 49.5cm (<1 centile, SDS-2.72). He has moderate intellectual disability with an IQ of 63 and no signs of autism spectrum disorder. Physical examination revealed short stature, thin build, and both hyper- and hypopigmentations. He had genu valgum, syndactyly, camptodactyly, *pectus excavatum* and spinal kypholordosis. Upon neurological examination, he had no muscle weakness. Deep tendon reflexes could not be provoked and there were no pathologic reflexes. He had mild truncal hypotonia. Myokymia or movement disorder could not be observed. Dysmorphic features included a sandal gap at both feet. He has a triangular face with a pointed chin.

## **FAMILY 2**

### **Family 2, II-1**

The individual is an 11-year-old boy who was referred to Medical Genetics for a history of developmental delays, bilateral hip dysplasia, abnormal electromyogram (EMG) with possible myopathy and mitochondrial cytopathy. He had normal early milestones. He sat unassisted at 6 months of age, was crawling and cruising before the age of one. The delays were noted at around one year of age. He receives speech therapy, occupational therapy, and physical therapy in school. He started to talk at the age of three years. He also has a history of fine motor delays. He has a history of frequent respiratory infections, ER visits and hospitalizations for that. He had an EMG that was an abnormal study with evidence for myokymic discharges creating continuous muscle fiber activity, changes in recruitment of voluntary motor unit activity suggestive of possible myopathy and in addition, bilateral moderately severe median neuropathy at the wrist (carpal tunnel syndrome). He had a muscle biopsy and mitochondrial respiratory chain enzyme analysis which demonstrated increased citrate synthase activity which per lab report was suggestive of mitochondrial proliferation; however, no deficiencies of respiratory chain enzymes were detected before or after correction for increased citrate synthase activity. The family history is notable for a younger brother who is 4 years old and with a history of speech delay and motor delay (he is not walking). The family history is also notable for consanguinity. Parents are first cousins. Neurological assessment depicted: cranial nerves III through XII are intact. His muscle strength in the upper limbs is normal except for mild weakness of the pectoralis major muscles bilaterally, and he has well developed muscles in the upper limbs. In the lower limbs, he has atrophy of the muscles below the knee. He walks with mild flexion of the knees. He is able to stand and take a few steps on his toes, but he is not able to stand or walk on his heels. Perhaps the tibialis anterior

and gastrocnemius muscles are weak. He has -2 hypoactive reflexes symmetrically, and he has bilateral flexor plantar responses. He withdraws to light touch and pinprick, and he also recognizes hot and cold sensations.

### **Family 2, II-2**

The individual is an 8-year-old product of mother's third pregnancy. There were no apparent fetal complications during the gestation, although the mother had achalasia of the cardia and as a result had rather reduced intake and indeed aspirated and experienced pneumonia subsequent to the delivery. This was an unplanned pregnancy. He was delivered by lower segment Cesarean section at term with a birth weight of 7 pounds. He had no perinatal complications including jaundice or respiratory difficulties. Mother became concerned at 1 year when he was not walking. He had x-rays of his hips which were normal and subsequently began physical, occupational, and speech therapy at 15 months of age. Mother notes that he has very good receptive abilities in both English and Arabic which the parents speak at home. He has had no difficulties with his chewing or swallowing. Mother has noticed that he has increasing stiffness over time. He has developed an unusual gait in which he walks with his knees flexed and slightly abducted. He had nerve conduction study (NCS) that showed a slowed peroneal motor conduction velocity with slowed distal latency, low-amplitude tibial motor response with low-amplitude sensory responses. The median palmar latencies were slightly prolonged compared with the ulnar palmar responses. F-waves were within estimate but were poorly formed, but repetitive discharges were noted on F-waves. There was no conduction block or temporal dispersion. Needle examination revealed continuous myokymic-like bursts of motor activity even with deep sedation. No fibrillation potentials or myotonic discharges were noted. There was rapid recruitment of short-duration complex motor unit potentials at most locations with more distal than proximal involvement. The

findings were interpreted as consistent with a neuromyotonic syndrome with myokymic discharges raising the possibility of channelopathy. There were no findings to suggest inflammation, muscle necrosis, vacuolization, or fiber splitting on muscle biopsy. The possibility of very mild median neuropathies at the wrist, right greater than left, was considered, although given the presence of a neuropathy, this interpretation must be made with caution. There has been no history of regression. He also had biopsy of bilateral vastus lateralis muscle and that showed slight denervation atrophy as well as type 1 fiber smallness in some fascicles. At neurological evaluation: His cranial nerves III through XII are intact. His muscle bulk and strength and tone on the upper limbs seem to be age appropriate. However, in the lower limbs, it is not possible to ascertain if his tibialis anterior or gastrocnemius muscles are strong or not, but he has more muscle bulk in those muscles compared to his brother's muscle bulk. Muscle stretch reflexes are -2, symmetric. He has flexor plantar response on the left. His right lower limb was in a cast after surgery for contractures correction. He feels light touch, hot and cold sensations.

### **FAMILY 3**

II-2 and II-3 are the second and third children of healthy, related parents (first cousins), there is a healthy elder brother.

#### **Family 3, II-2**

This is a male subject who was 7 years old at last examination. He was born after an uneventful pregnancy, except an elevated risk for Down syndrome on maternal first trimester screening. He was born at full term, birth weight was 3720g, height was 52 cm and OFC was 35 cm. He had global developmental delay, with mild gross motor delay, he started catching objects at 6 months, sitting at 9 months, walking at 18 months, and had difficulties with fine motor skills. Speech was

delayed and he had learning disabilities with special education needs. His feet were normal at birth, then in the first years he was seen for flat valgus feet, that evolved into a bilateral varus deformity needing orthopaedic treatment. He had no seizures. Cerebral and medullar MRI were normal, as well as cardiac and abdominal ultrasound. Hearing and ophthalmological examination were also normal. ENMG was normal. Clinical examination showed normal weight, height and OFC. He had a small mouth with limited opening, a central atrophy of the tongue, and fasciculations of the tongue and of the face, especially around the mouth and eyes. He had talipes equinovarus, and absent deep tendon reflexes on four limbs.

### **Family 3, II-3**

This individual is a female who was 4 years 7 months at last examination. She was born at full term, after an uneventful pregnancy, birth weight was 3560g, length at 51 cm and OFC at 36 cm. She had global developmental delay, held her head at 4 months, and started walking at 18 months. Speech was delayed and she had learning disabilities, but less than her brother. At 18 months, bilateral varus deformity of the feet was noted, and are under orthopaedic treatment. She had no seizures. Cerebral and medullary MRI were normal, as well as cardiac and abdominal ultrasound. Hearing was normal, and she had mild strabismus. EMG showed fasciculations of the muscles of the mouth floor, and absent late F responses. Clinical examination showed normal weight, height and OFC. She had a small mouth with limited opening, a central atrophy of the tongue, and fasciculations of the tongue and of the face, especially around the mouth and eyes. She had talipes equinovarus, more severe than her brother, and absent deep tendon reflexes on four limbs.

### **FAMILY 4**

Case report of this family was previously published (as Family 10 in Froukh 2017).<sup>1</sup>

## **FAMILY 5**

### **Family 5A, II-1**

This is an 11-year-old boy who was referred to genetics clinic along with his sister (Family 5, II-2) with intellectual disability, and FTT. The condition started at age of 2 years with an episode of recurrent abdominal distention and failure to gain weight followed with gastroenterology team and diagnosis of celiac diseases were raised. Later parents noticed some delay in his cognitive skills and speech at 4 years of age. Perinatal history was unremarkable apart from club foot which was corrected surgically at 4 years of age. Parents are not cousins, they have another affected daughter with the same phenotype, and another 5 healthy children. His motor skills were appropriate for age; however, his cognitive and speech milestones were significantly delayed. He can express small sentences which are partially understood by the family, and is able to follow simple commands. Last IQ assessment done was 59. Physical examination unremarkable but the growth parameters for height and weight are below the 3<sup>rd</sup> centile (130 cm and 26.6 kg). All investigations came negative including, lipid profile, creatine kinase, ammonia, lactic acid, homocysteine, plasma amino acid, urine organic acid, carbohydrate Deficient Transferrin, and acylcarnitine profile. Karyotype revealed normal male 46,XY. Additionally, Electromyography (EMG) and nerve conduction study (NCS) were normal.

### **Family 5A, II-2**

The affected sister is 9 years old with similar presentation of speech delay, intellectual disability and poor school performance. Her physical examination was unremarkable however her growth parameters were below 3<sup>rd</sup> centile for age (125 cm and 25.5 kg). metabolic and genetic

investigations were unremarkable including normal female 46,XX. Her EMG and NCS are normal, and last IQ assessment was 55.

### **Family 5B, II-1**

This individual is a 13-year-old female with intellectual disability and poor school performance. She also showed delays in her speech, her first word was at age of 3 years. Now she can say few words with poor articulation and with pronunciation difficulties. Physical examination was unremarkable however her growth parameters were below 3<sup>rd</sup> centile for age. She is wearing eyeglasses for strabismus. Laboratory investigations were unremarkable including normal female 46,XX. MRI brain done and showed normal records. Her last IQ assessment was 69.

### **Family 5B, II-2**

This is a 9-year-old individual who was seen as a case of mild cognitive delay and celiac disease. Family first concern was at the school age. She was found to have learning difficulty when she attended the first grade, she faced significant difficulty in the learning. IQ test was done the result was 66. She was diagnosed to have celiac disease two years back after slow growth. Perinatal history was unremarkable. Her motor development was appropriate for age, although in her cognitive and speech skills delayed. She started to speak at 3 years of age, currently she is at 2<sup>nd</sup> grade at elementary school. Parents are first cousins, another four daughters and one son. One of the daughters has similar presentation with learning difficulty. Physical examination unremarkable but the growth parameters for height and weight are below the 3<sup>rd</sup> centile (115 cm and 18.3 kg). All investigations came negative including, lipid profile, creatine kinase, ammonia, lactic acid, homocysteine, plasma amino acid, urine organic acid, and acylcarnitine profile. Karyotype revealed normal female 46,XX. Additionally, array CGH was negative.



## **FAMILY 6**

II-6 (BAB12529), II-1 (BAB12532), and II-5 (BAB12533) are three male siblings born to a consanguineous family from Iraq. They were normal until three to seven months of age when they started showing tremulous movement around mouth and ocular area with posturing and twisting of hands and feet. All three siblings had delayed motor milestones and mild (subjects BAB12529 and BAB12533) to severe cognitive delays (subject BAB12532). Frequent falls and occasional complaints of muscle pain were often observed. Physical examinations revealed facial myokymia in periocular and perioral region in all three siblings. Moreover, finger deformities, inverted posturing of feet, increased tone and reduced deep tendon reflexes (DTR) were consistently present. Their histories and examinations are detailed next.

### **Family 6, II-1 (BAB12532)**

This is the eldest sibling of the three affected males in family 5 and is currently 13 years old. He was born post-term. His history was similar to his siblings, except that his clinical features appeared earlier with onset of occasional tremulous movement around eyes and oral cavity at 3 months of age. He has moderate to severe cognitive impairment and sometimes complains of muscle pain. His family reports continuous improvement in motor skills with age. On examination, he appeared alert and non-dysmorphic. His cranial nerves were intact, and he had normal muscle power. He was hypertonic throughout and had areflexia except for the supinator. He was also noted to have finger deformities/contractures, bilateral varus deformity, inverted posturing of feet. His workup included a normal brain MRI (at 5 years of age) and a normal routine awake and asleep electroencephalogram (EEG) [at 11 years of age].

### **Family 6, II-5 (BAB12533)**

This subject is currently 6 years old. He was born at term and was also developing well until 7 months of age when he gradually developed sudden irritability, posturing/twisting of limbs with tremulous movements. He later showed signs of motor delays and occasional falls and started complaining of muscles pain. Currently, he has mild cognitive impairment, and is partially toilet trained. He can walk independently but with frequent falls. He has exaggeration of ambulation and weakness during febrile illness. He has history of operated diaphragmatic hernia. On examination, he was alert and non-dysmorphic. He had mild abdominal distension. Flickering movement at the perioral area, hypertonia of the limbs, flexion deformities/contractures of fingers, bilateral varus and planter flexion/inversion posturing of feet were also noted. The individual also showed absent DTR except for supinators. His muscle power was 4+/5 throughout. Brain MRI at 5 years of age was normal. NCS of the bilateral median, ulnar, sural, common peroneal and tibial nerves performed at 6 years of age was normal. EMG study however showed evidence of spontaneous activity in the form of fibrillations, positive sharp waves, myokymia, and fasciculations involving multiple cranial, cervical, lumbosacral segments suggestive of an early presentation of motor neuron disease. The CPK level was normal.

**Family 6, II-6 (BAB12529)**

This subject is currently 3 years old. He was born at term and was developing well until 7 months of age when he gradually developed tremulous movement around mouth and ocular area with posturing and twisting of hands and feet and complaints of muscle pain. His family also reports motor developmental delay (he sat at 18 months of age and walked at 2 years of age). Currently, he has mild cognitive impairment. He walks independently yet falls frequently and runs with difficulty. Additionally, he can go up and down stairs. He often complains of leg pain and weakness, especially in the morning. His family noticed that he becomes weaker and non-

ambulatory during febrile illnesses. On examination, he appeared alert and had no dysmorphic features. He had mild abdominal distension and umbilical hernia. Flexion posturing of fingers and planter flexion and inversion posturing of feet were also noted. He had increased tone throughout and absent DTRs except for left biceps and supinator. He had good muscle power and was able to move all extremities without difficulty.

## **FAMILY 7**

### **Family 7, II-1**

This 16-year-old girl was born to non-consanguineous parents without asphyxia as a first child after an uneventful 39-week gestation. The birth weight, height, and occipito-frontal circumference (OFC) were 3,516 g, 53.0 cm, and 34.0 cm, respectively. Her father felt generalized stiffness at suffering from influenza A. She developed generalized complex partial seizures with or without tonic clonic convulsion from 8 months of age. Interictal EEG was normal. She was diagnosed as epilepsy and took Zonisamide (ZNS). Thereafter, seizures were controlled, and ZNS was stopped at 1y 9m. She was noticed to fist her hands all the time after about 6 months of age. She underwent an operation for umbilical hernia at 1y 1m, and her fisting thereafter exacerbated. She held the head at 3 months, rolled over at 6 months, sat alone at 7 months, crawled at 7 months, crept at 10 months, stood with support at 10 months, and walked with support at 16 months; she later walked at 34 months with flexed and abducted hips and flexed knees. At 1y 6m, she was neurologically assessed firstly. Her mouth was closed tightly, and her hands were fisted; these were observed to persist during sleep. The hips were flexed and abducted, and knees were flexed. The muscle of the extremities was stiff but is not considered to be spastic by passive stretch. Deep tendon reflexes were not elicited. After 4y 11m, the subcutaneous areas around the corner of the mouth or at the superior eyelid had been observed to twitch; the mother said that a worm moved

here. While she caught a fever from upper respiratory tract infection at 8y 1m, she was motionless with stiffness of the whole body and continued sleeping for four days; the mother said that she was like a stone. While she suffered from varicella at 9y 0m, she was motionless with stiffness for several hours without sleeping. After 11y 10m, she episodically felt vertigo with freezing. Her intellectual development was assessed to be delayed moderately; the intelligence quotient examined by Wechsler Intelligence Scale for Children-Fourth edition at 7y 10m was 41. Electromyography exhibited myokymic discharges at rest. The brain MRI implied mild frontal hypoplasia. Mexiletine was definitely effective, and acetazolamide was in a degree effective for reducing stiffness. Sultiame was effective against episodic vertigo.

## **FAMILY 8**

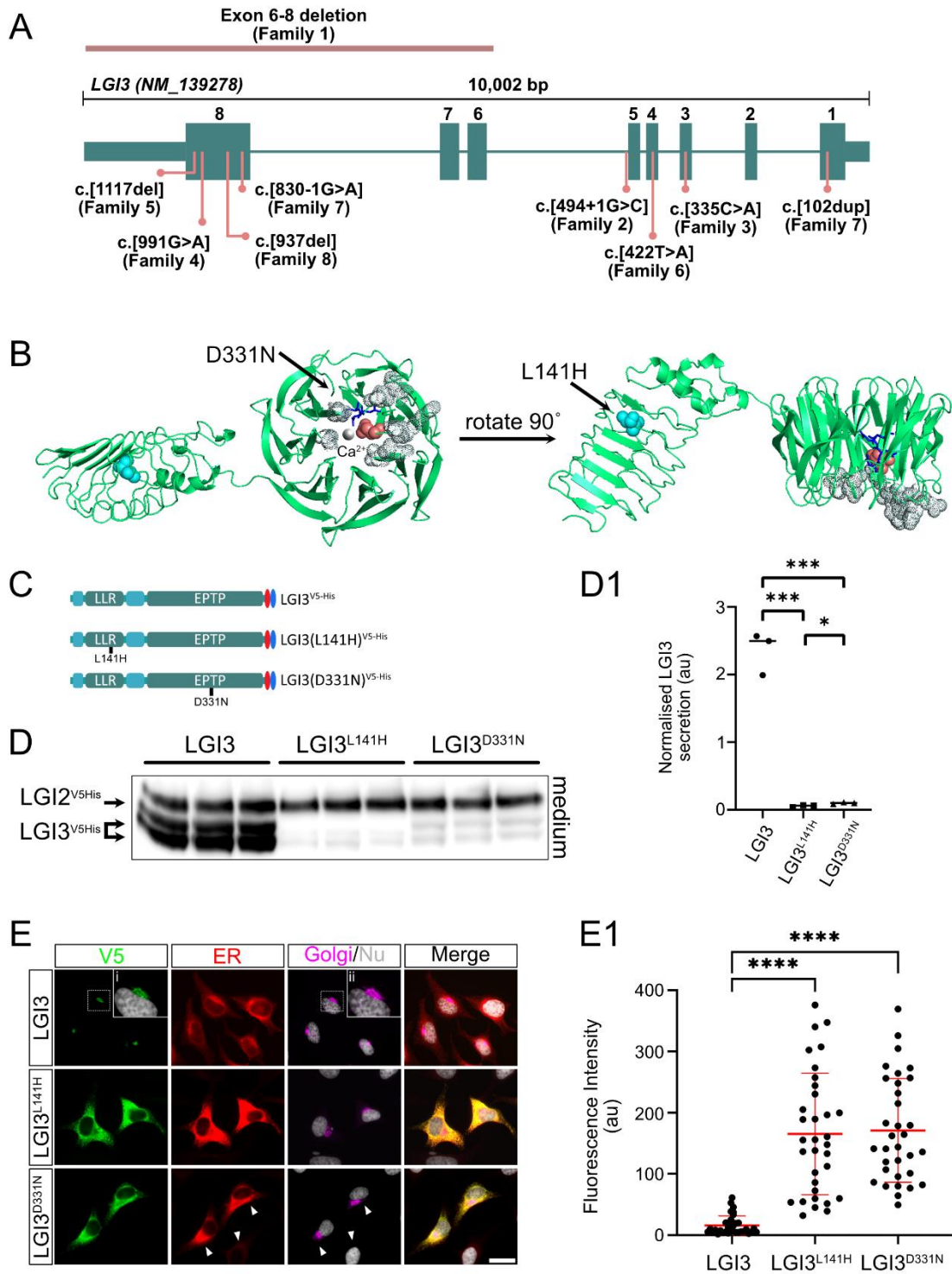
### **Family 8, II-2**

This 13-year-old male was born spontaneously with no asphyxia as the second child of consanguineous parents whose fathers were brothers. He showed clubfeet and mild contractures in both hands and forearms at birth. His growth and motor development were normal during infancy, but his mother noticed tongue fasciculation and perioral myokymia. Brain MRI and electrophysiological findings (auditory and somatosensory evoked potential and blink reflex) at 6 years of age were unremarkable, when he received orthopedic surgery for clubfeet. At the age of 14 years, he was thin (158.6cm, 46.5kg) and myopathic with thin face, long philtrum and open mouth (Fig. 1P). Tongue fasciculation and perioral myokymia were still present. He could chew and swallow well, but showed dysarthria, such as a conversion of sounds [s], [k] to [t], difficulty in tongue protrusion, and perioral hypersensitivity and extreme contraction around the mouth leading to the difficulty in tooth brushing. Drooling was not apparent, but he slurped saliva frequently. His hip joints were externally rotated, and postoperative *pes planovalgus* was present.

As for upper limbs, only adduction of bilateral thumbs was limited. He could run slowly and could stand over 20 seconds and jump on each leg. He had a severe intellectual disability and was unable to read Hira-kana, which is the simplest form of Japanese letters, but could understand and follow some plain orders and spoke short sentences. He showed hyperactive type of attention-deficit hyperactivity disorder and mild autistic spectrum disorder. He had no seizure episodes ever.

# SUPPLEMENTAL FIGURES

Figure S1



**Figure S1. Gene and protein structures of *LGI3* variants and their cell biological expression**

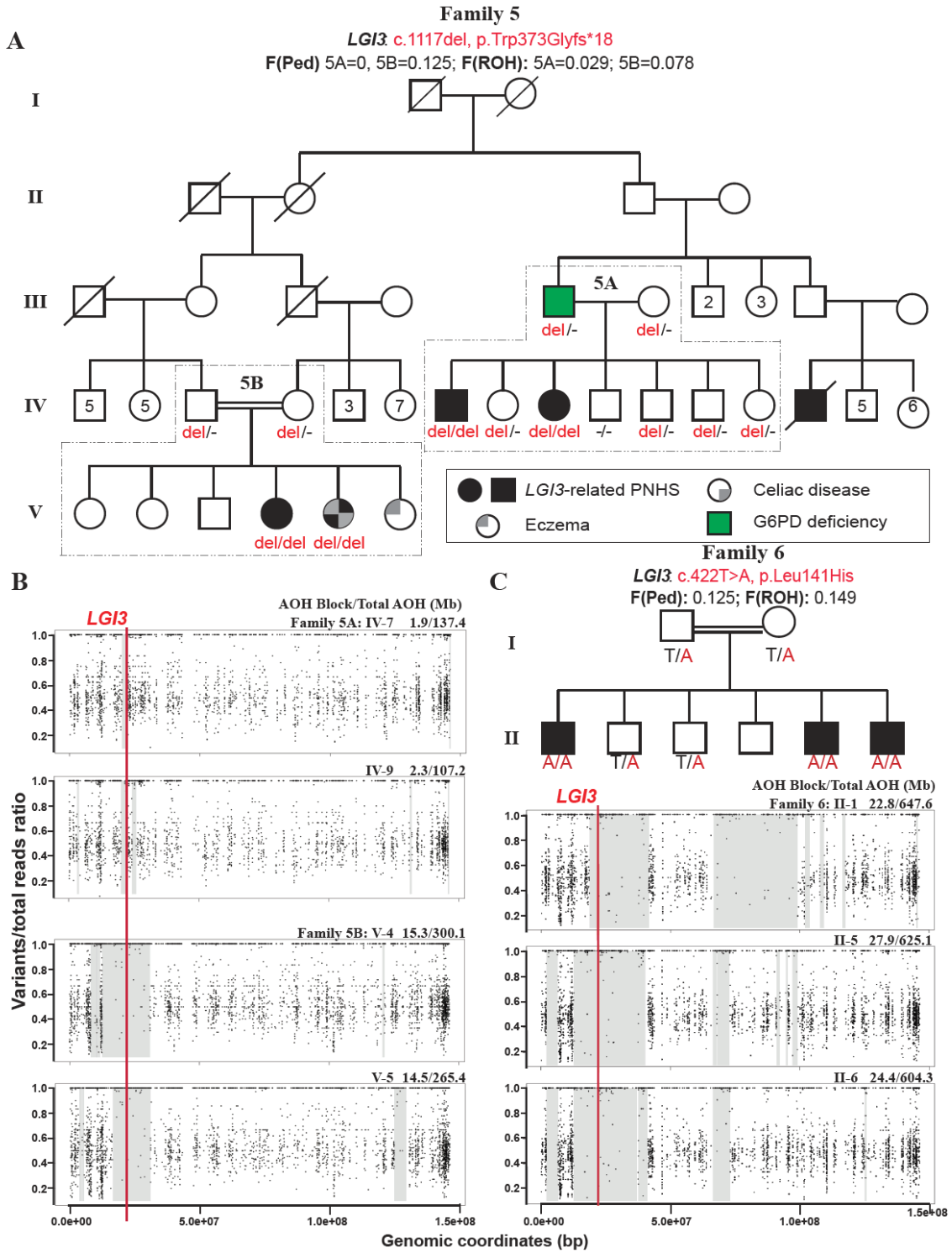
- (A) Locations and distribution of all identified variants on a linear gene structure of *LGI3* (NM\_139278). The green-grey boxes represent exons 1-8 of *LGI3*. The identified variants in this study are marked by red rounded tip arrows.
- (B) A structural model of the *LGI3* protein was generated using the Phyre2 web application using the structure of *LGI1* as a template (PDB file C5Y31B, 53% identity, 100% confidence) and visualized using PyMol.<sup>2; 3</sup> The position of the altered amino acid residues encoded by the two identified missense variants in this study c.422T>A; p.(Leu141His) and c.991G>C; p.(Asp331Asn) are marked by space-filling renditions in light blue and red respectively. The amino acids involved in ADAM22 interactions are indicated with stipled spheres and are located on one side of the EPTP domain. The missense variant L141H is located in the fourth LRR repeat of the LRR domain in a highly conserved patch that is identical in all *LGI* proteins. The mutation is marked as deleterious by both the PolyPhen2 as well as Phyre2 algorithms. The D331N mutation is located in the hydrophobic core of the EPTP domain and is involved, together with three other negatively charged amino acids (dark blue sticks), in coordinating a Ca<sup>2+</sup> ion. The conserved nature of these charged amino acids, present in all *LGI* proteins, suggest Ca<sup>2+</sup> coordination is an important feature of the EPTP domain structure. Indeed, Polyphen2 and Phyre2 mark this mutation as highly unfavourable.
- (C) Schematic depiction of expression cassettes of the mouse *LGI3* protein and the L141H and D331N *LGI3* mutant proteins. The *LGI3* proteins carry a V5-tag and 6xHis tag at their carboxy terminal end.
- (D) *LGI3*, *LGI3*<sup>L141H</sup> and *LGI3*<sup>D331N</sup> proteins were transfected into HEK293T cells, in triplicate, and recovered from tissue culture medium using Ni-NTA agarose affinity precipitation.

Western blotting revealed that whereas LGI3 is readily expressed and secreted, LGI3<sup>L141H</sup> and LGI3<sup>D331N</sup> are not. Levels of secretion of the LGI3 proteins were quantified using ImageJ and demonstrated a statistically significant drop in secretion of both mutant proteins. Interestingly, the D332N mutation is less severe, and some secretion of mutant protein is observed. Both mutant proteins still bind to ADAM22 and ADAM23 (not shown).

(E) HEK293T cells were transfected with LGI3, LGI3<sup>L141H</sup> and LGI3<sup>D331N</sup> and a Golgi-Scarlet expression cassette. Cells were stained for LGI3 (V5 in green) and the ER (KDEL in red). Purple staining reveals the structure of the Golgi apparatus in transfected cells. Note that the KDEL signal in LGI3 mutant expressing cells is highly increased (white arrowheads point at two transfected cells and one non-transfected cell in the same field of view) suggesting that the mutant proteins elicit a strong ER stress response. LGI3 expression levels (V5 immunofluorescence signal) were quantified, normalized to Scarlet Golgi signal, and plotted in E1. Magnification bar = 15  $\mu$ m.



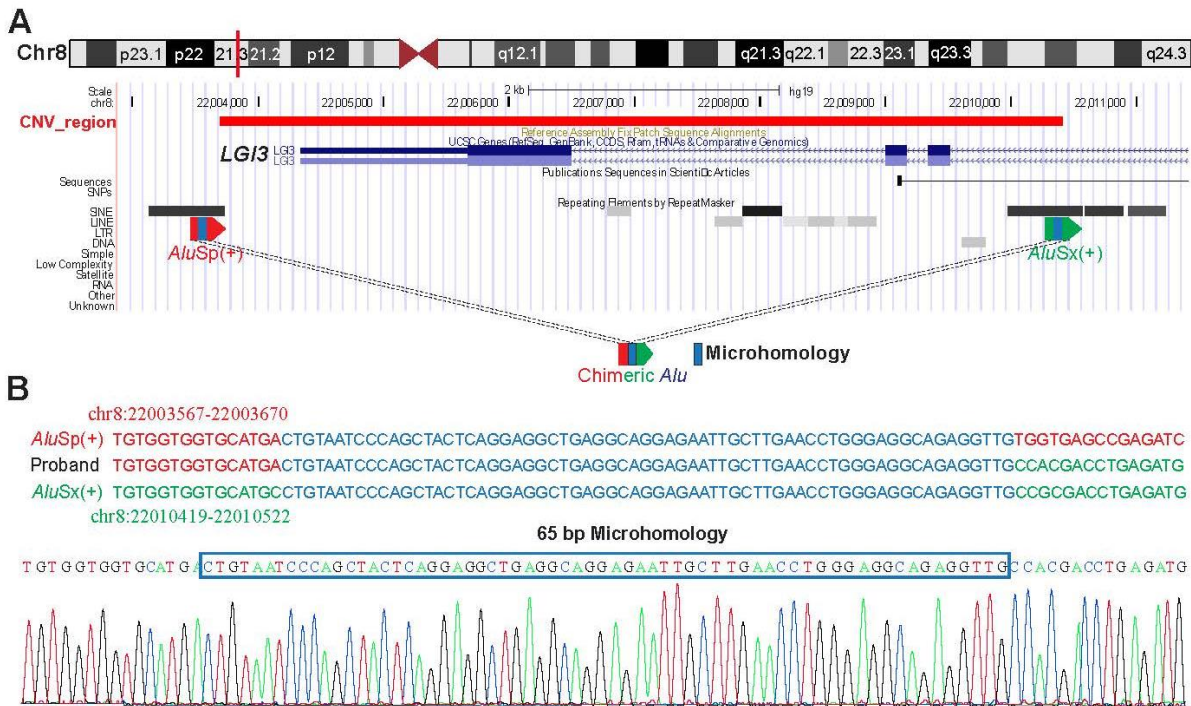
Figure S2



**Figure S2. Extended multigeneration pedigrees of Families 5 and 6 and Absence-of-Heterozygosity (AOH) blocks of affected individuals.**

- A. The extended multigeneration pedigrees of Family 5 showing both branches of the family (Family 5A and Family 5B). As can also be seen, there is a deceased paternal male cousin of the affected children in Family 5A with a reported phenotype (of global developmental delay, intellectual disability and distal contractures) resembling those of in the spectrum of *LGI3*-related peripheral hyperexcitability syndrome. However, the molecular diagnosis of this deceased cousin could not be confirmed due to unavailability of a biological specimen.
- B. Absence of Heterozygosity (AOH) blocks around the *LGI3* variant in the affected individuals from Family 5A and 5B. The position of the *LGI3* variant is marked by a red line. As seen, there is an 1.9 Mb overlapping AOH block denoting a shared haplotype.
- C. Pedigree of Family 6 followed by the AOH blocks around the *LGI3* variant in the three affected siblings in the family. The position of the *LGI3* variant is marked by a red line. As seen, the variant falls within a large AOH ranging between 22.8-27.9 Mb.

**Figure S3**



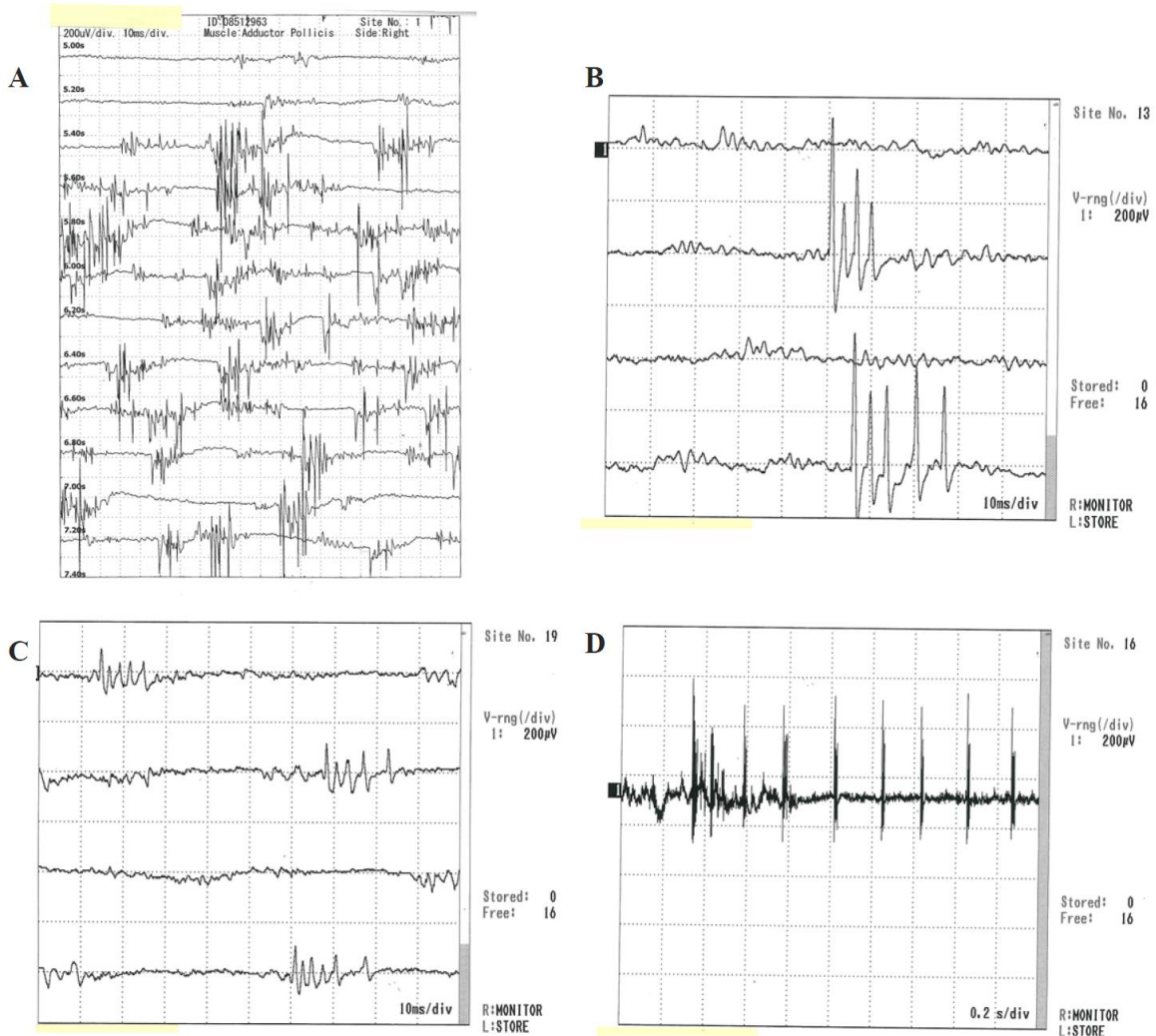
**Figure S3. Breakpoint junction analysis of the multi-exonic intragenic deletion of *LG13* in Family 1 derived from an *Alu/Alu* mediated genomic rearrangement (AAMR)**

(A) A genomic view of exons 6 to 8 of *LG13* from the UCSC genome browser (hg19) shows multiple flanking *Alu* repeats within the targeted genomic interval. The SINE element pair, *AluSp* and *AluSx*, involved in this AAMR generating the exonic deletion allele are marked with a red and green arrow, respectively, with the microhomology marked in blue. The deletion allele (CNV region) is demarcated by red horizontal bold line.

(B) Breakpoint junction analysis via Sanger sequencing reveals a 6852 bp deletion resulting in a chimeric *Alu* with the breakpoint junction mapping within 65bp microhomology. The three sequences from top to bottom represent the reference *AluSp* sequence (colored in red), the sample “chimeric” sequence and the *AluSx* sequence (colored in green). The region of

microhomology is colored in blue. The bottom image represents Sanger tracing of the breakpoint junction with the 65 bp of microhomology marked with a blue box.

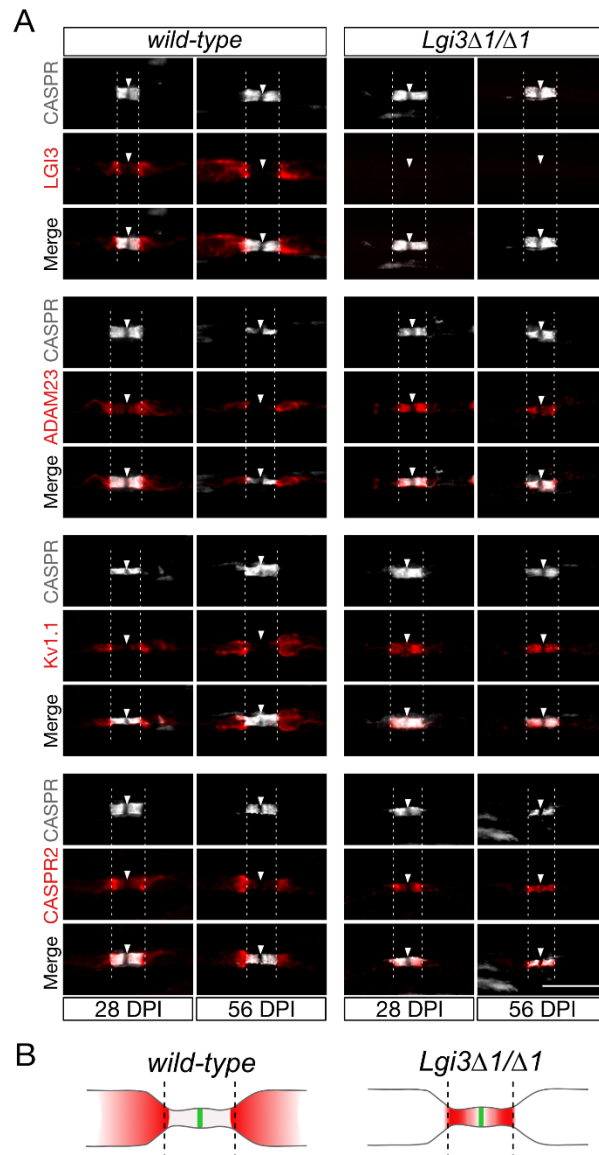
**Figure S4**



**Screenshots of the Electromyogram (EMG) recording of Family 7, II-1.**

EMG performed with a concentric needle electrode of right adductus pollicis muscle showing myokymic discharge at rest (Voltage: 200µVol/Division; Sweep speed in Panels A-C is 10ms/division and in Panel D is 200ms/division).

Figure S5



**Figure S5 Reassembly of Kv1 channel complexes in remyelinating nerves.**

A. Wild-type and *Lgi3 $\Delta 1/\Delta 1$*  animals were subjected to crush-injury induced demyelination of the Sciatic nerve. The reassembly of Kv1 channel complexes was examined during remyelination of the injured nerves at 28 and 56 days post injury (DPI) Representative images of the node of Ranvier from immune-stained teased sciatic nerve fibers of wild-

type (left panel) and *Lgi3<sup>Δ1/Δ1</sup>* (right panel) mice are shown. Single and merged stains are shown for each image. All images include CASPR (CNTNAP1: in grey) and one of the following: LGI3, ADAM23, Kv1.1 (KCNA1), CASPR2 (CNTNAP2) in red. Scale bar=10μm.

- B. Schematic representation of the position of the Kv1 channel complexes (in red) in remyelinated axons (56 DPI) to illustrate the failure to evict the complexes from the paranodal domain in the absence of LGI3. The position of the node is indicated in green.

## **SUPPLEMENTAL TABLES**

**Table S1. Other clinical features and investigations in the 16 affected individuals with biallelic deleterious variants in *LGI3***

Family & Individual	Clinical Features					Clinical Investigations				Others
	ASD	ADHD	Abnormal muscle tone	Distal deformities	Seizures	NCS	EMG	Muscle Biopsy	Brain MRI	
<b>F1, II-3</b>	-	-	+ (calve and truncal hypotonia)	+ (knee contractures)	-	Normal	N/A	N/A	Small hypophysis	Progressive microcephaly (-5 SD); kyphosis; FTT (-4 SD); GH deficiency due to <i>GHRHR</i> homozygous pathogenic variant, discrete occasional epileptiform discharge on EEG
<b>F2, II-1</b>	-	-	+ (decreased in LL)	+ (knees, hips, and ankles contractures)	-	N/A	Continuous myokymic discharge, changes in recruitment	Increased citrate synthase activity. No deficiency in RC enzymes	N/A	Bilateral hip dysplasia
<b>II-2</b>	-	-	+ (stiff)	+ (knees, hips, and ankles contractures)	-	Slow CV and prolonged DL (motor peroneal), reduced Amp (tibial); Reduced Amp (tibial), slightly prolonged DL (median palmar compared to ulnar palmar). Poorly formed F-wave with repetitive discharges.	Continuous myokymic discharge. No Fibs or myotonic discharges. Rapid recruitment of short-duration complex MUAP (distal > proximal). Ulnar neuropathy. Median neuropathy at wrist	Slight denervation atrophy; type 1 fiber smallness in some fascicles	N/A	
<b>F3, II-2</b>	+	-	-	+ (talus valgus; bilateral club feet; tapered fingers with	-	N/A	Normal	N/A	Normal	

<b>II-3</b>	+	-	-	spatulated tips) + (talus valgus; bilateral club feet; mild stiffness of finger joints with no evident camptodactyly; adducted thumbs with preserved mobility; mild retraction of toes)	-	N/A	Fasciculations of floor of mouth and absent F response	N/A	Normal	
<b>F4, II-1</b>	+	+	N/A	-	+	N/A	N/A	N/A	N/A	Epileptiform discharge on EEG
<b>II-2</b>	+	-	N/A	-	-	N/A	N/A	N/A	N/A	
<b>F5A, II-1</b>	-	+	-	+	-	Normal	Normal	N/A	N/A	
<b>II-2</b>	-	+	-	+	-	Normal	Normal	N/A	N/A	pectus carinatum
<b>F5B, II-1</b>	-	+	-	+	-	N/A	N/A	N/A	Normal	Anxiety
<b>II-2</b>	-	-	-	+	-	N/A	N/A	N/A	N/A	Celiac disease; Eczema
<b>F6, II-1</b>	-	-	+(increased)	+ (finger deformities/contractures, bilateral varus deformity, inverted posturing of feet	-	Normal	N/A	N/A	Normal	Frequent falls and occasional muscle pain; normal asleep only EEG
<b>II-5</b>	-	-	+(increased)	+ flexion deformities/contractures of fingers,	-	Normal	Myokymic discharge, Fibs, PSW, and fasciculations (multiple cranial,	N/A	Normal	Frequent falls and occasional muscle pain; mild abdominal distension and



<b>II-6</b>	-	-	+(increased)	bilateral varus and planter flexion/inversion posturing of feet + (deformities/contractures of fingers, and foot inversion)	-	N/A	N/A	N/A	N/A	cervical, and lumbosacral segment)	operated diaphragmatic hernia; normal CPK level
<b>F7, II-1</b>	+(Mild)	-	+(stiff)	+ (flexion and abduction of hips, flexion of knees and fisted hands)	+	Normal	Myokymic discharge at rest	N/A	Suspected mild frontal hypoplasia		Ataxia; Hypokinesia; Operated umbilical hernia; Normal EEG
<b>F8, II-2</b>	-	-	+(hypotonia)	+	-	N/A	N/A	N/A	Normal		chr16q13.11 microduplication; operated R diaphragmatic hernia
<b>Total</b>	5/16	4/16	7/13	14/16	2/16						

**Abbreviations:** ADHD, attention deficit hyperactivity disorder; Amp; amplitude; ASD, autism spectrum disorder; CPK, creatine kinase; CV, conduction velocity; DI, distal latency; EEG, electroencephalography; EMG, electromyography; Fibs, fibrillations; FTT, failure to thrive; GH, Growth hormone; LL, lower limbs; MRI, magnetic resonance imaging; N/A, not available; NCS, nerve conduction study; PSW, positive sharp waves; RS, respiratory chain.

**Table S2. Detailed motor and speech developmental milestones in all 16 affected individuals with biallelic *LG13* variants**

Family & Individual	Age at last examination	OFC at birth	Motor development					Speech development			
			Head control	Grabbing	Sitting without support	standing without assistance	Walking without assistance	Babble repetitive syllables (ba. ba. ba)	Says a few words ("mama")	Points to an object or picture	Speaks 50 words and understands more
<b>F1, II-3</b>	7yo	34cm (-1.23 SDS)	N/A	N/A	12 mo	N/A	N/A	19 mo	20 mo	N/A	
<b>F2, II-1</b>	9yo	N/A	N/A	N/A	6 mo	N/A	4 yo	15 mo	3 yo	N/A	Began more significant vocabulary at age 3y. Started putting three-to-four-word phrases together at 6 y
<b>II-2</b>	6yo	N/A	N/A	N/A	6 mo	N/A	5 yo	N/A	6 yo	N/A	Does not speak, but knows letters, numbers, and colors. He can imitate sounds.
<b>F3, II-1</b>	7yo	35 cm	N/A	6-7 mo	9 mo	N/A	18 mo	N/A	5 yo	N/A	says words but difficult to understand. Comprehends well.
<b>II-2</b>	4yo 7mo	36 cm	4 mo	N/A	11 mo	N/A	18 mo	N/A	N/A	N/A	yes (better than her brother, however, speaks only in her native language not in French)
<b>F4, II-1</b>	22yo	N/A	delayed	N/A	9 mo	delayed	N/A	18 mo	N/A	N/A	6 years with stammer
<b>II-2</b>	20yo	N/A	delayed	N/A	8 mo	delayed	N/A	16 mo	N/A	N/A	N/A

<b>5A, II-1</b>	11yo	N/A	N/A	N/A	N/A	delayed	3 yo	N/A	N/A	N/A	Speaks in sentences with unintelligibility
<b>II-2</b>	9yo	N/A	N/A	N/A	N/A	delayed	2 yo	N/A	N/A	N/A	Speaks in sentences with unintelligibility
<b>F5B, II-1</b>	13yo	N/A	Normal age	N/A	Normal age	Normal age	1 yo	Delayed	Delayed	N/A	She can say 5 words now with poor articulation and difficult pronunciation
<b>II-2</b>	9yo	N/A	Normal age	N/A	Normal age	Normal age	1 yo	3 yo	3 yo	N/A	She can say 3 words sentences with pronunciation problems
<b>F6, II-1</b>	13yo	N/A	2 yo	3 yo	2 yo	2.5 yo	3 yo	2.5 yo	2.7 yo	N/A	4 yo started talking in sentences at 5 yo
<b>II-5</b>	6yo	N/A	19 mo	30 mo	30 mo	3 yo	3.5 yo	N/A	2 yo	N/A	
<b>II-6</b>	3yo	N/A	at expected age	at expected age	18 mo	20 mo	2 yo	> 1 yo	> 1 yo	18 mo	3 yo
<b>F7, II-1</b>	12yo 10mo	34.0cm	3 mo	N/A	7 mo	2yo 6mo	2 yo 10 mo	N/A	2 yo 3 mo	N/A	5yo 2mo
<b>F8, II-2</b>	13yo	34.8cm	4 mo	N/A	N/A	22 mo	22 mo	N/A	N/A	N/A	N/A

Abbreviations: N/A, not available; OFC, Occipitofrontal Circumference; mo, months; yo, years old

# SUPPLEMENTAL METHODS

## **Variant annotation methods and exome sequence analysis**

**Family 1** - Quad-Exome sequencing of the index case, the unaffected sibling and the parents was performed using a SureSelect Human All Exon 60Mb V6 Kit (Agilent) for enrichment and the NovaSeq6000 (Illumina) platform for sequencing. An average of 135,888,843 reads were produced per sample and aligned to the UCSC human reference assembly (hg19) with BWA v.0.5.8.1. More than 98% of the exome was covered at least 20× and the average depth-of-coverage was more than 126×. Single-nucleotide variants (SNVs) and small insertions and deletions were detected with SAMtools v.0.1.7. Copy number variations (CNVs) were detected with ExomeDepth<sup>4</sup> and Pindel<sup>5</sup>. Variant prioritization was performed based on an autosomal recessive (MAF <0.1%) and autosomal dominant (*de novo* variants, MAF <0.01%) inheritance models.

**Family 2**- Exome sequencing was performed on genomic DNA extracted from all samples submitted. The exome was captured utilizing the SureSelect Human All Exon V5+UTR kit from Agilent Technologies. Sequencing was performed on an Illumina HiSeq 4000 Paired-end 101 base-pair reads were aligned to a modified human reference genome (GRCh37/hg19) using Novoalign (Novocraft Technologies, Malaysia). Sequencing quality was evaluated using FastQC ([www.bioinformatics.babraham.ac.uk/projects/fastqc/](http://www.bioinformatics.babraham.ac.uk/projects/fastqc/)). All germline variants were jointly called through GATK Haplotype Caller and followed by PhaseByTransmission to get phasing information.<sup>6</sup> Each variant was annotated using the BioR Toolkit,<sup>7</sup> and subsequently evaluated for clinical relevance by a team of scientists. Genome\_GPS v4.0 (formerly named as TREAT)<sup>8</sup> is a comprehensive secondary analysis pipeline for Exome/Whole Genome/Custom Capture sequencing data at Mayo Clinic. FASTQ files were aligned to the hg19 reference genome using

bwa-mem (VN:V7.10) with the default options. Realignment and recalibration was performed using GATK (VN:3.4-46)<sup>6</sup> best practices version 3 for each family separately. Multi-sample Variant calling was performed using the GATK (VN:3.4-46) Haplotype Caller and variants are filtered using variant recalibration Variant Quality Score Recalibrator (VQSR) for both SNVs and INDELS. Identified variants were annotated using BioR<sup>7</sup> framework with functional features, impact prediction, and clinical significance using CAVA, ClinVar, HGMD, and ExAC population frequencies. CNVs are detected using PatternCNV.<sup>9</sup>

**Family 3-**Exome sequencing was performed on the proband. Genomic DNA was extracted from peripheral blood lymphocytes using a Qiasymphony DNA Midi Kit (Qiagen). A SeqCap EZ MedExome Library kit (NimbleGen, Roche Sequencing) was used for genomic capture. A NextSeq 500 Sequencer was used for massively parallel sequencing. Raw data was analyzed with in-house annotation and analysis pipelines. For data analysis, raw reads were mapped to the human genome reference-build hg19 using the Burrows Wheeler Aligner (BWA MEM v0.717) alignment algorithm (H. Li and Durbin, 2010). The resulting binary alignment/map (BAM) files were further processed by Genome Analysis Tool Kit HaplotypeCaller (GATK HC v3.8) (Van der Auwera et al., 2013). The VCF files are then annotated on Snpeff version 4.3T (Cingolani et al., 2012). The variants are finally identified according to in house criteria. Only coding non synonymous and splicing variants were considered. Variant filtration was conducted according to the transmission mode (*de novo*, autosomal recessive and X-linked), frequency of the variant in the gnomAD database and to our in-house database of n = 866 exomes.

**Family 4-** Exome sequencing and analysis were performed as previously published in Froukh, 2017.<sup>1</sup>

**Family 5-** Exome sequencing and analysis were performed as previously published in Bertoli-Avella et al.<sup>10</sup>

**Family 6** – Pentad exome sequencing was implemented for the three affected siblings and their parents from Family 6 and was performed at the Human Genome Sequencing Center (HGSC) at Baylor College of Medicine (BCM) through the Baylor-Hopkins Center for Mendelian Genomics (BHCMG) initiative as previously described.<sup>11-13</sup> At HGSC, sequencing was performed using an Illumina dual indexed, paired-end, pre-capture library per manufacturer protocol with modifications as described in the BCM-HGSC protocol (<https://www.hgsc.bcm.edu/content/protocols-sequencing-library-construction>). Libraries were pooled and hybridized to the HGSC VCRome 2.1<sup>14</sup> plus custom Spike-In design according to the manufacturer's protocol (NimbleGen) with minor revisions. Paired-end sequencing was performed with the Illumina NovaSeq6000 platform with a sequencing yield of 12.2 Gb. Ninety-eight percent of targeted exome bases covered were sequenced to a depth-of-coverage of 20X or greater in all samples. The average depth of coverage for all samples was 119x. Illumina sequence analysis was performed using the HGSC HgV analysis pipeline,<sup>15; 16</sup> which moves data through various analysis tools from initial sequence generation to annotated variant calls (SNPs and intra-read in/dels). In parallel to the exome workflow, a SNP Trace panel was generated for final quality assessment. This included orthogonal confirmation of sample identity and purity using the Error Rate In Sequencing (ERIS) pipeline developed at the HGSC Using an “e-GenoTyping” approach, ERIS screens all sequence reads for exact matches to probe sequences defined by the variant and position of interest. A successfully sequenced sample must meet quality control metrics of ERIS SNP array concordance (>90%). Rare variant family-based exome analysis was performed as previously described.<sup>13</sup> The minor allele frequency of gene variants was obtained from gnomAD<sup>17; 18</sup> and our

in-house generated exome database of ~13,000 individuals at the BCM-HGSC. Variants were confirmed via Sanger sequencing.

**Families 7 and 8-** Trio exome sequencing was performed on both probands and unaffected parents from Family 7 and proband-only exome sequencing was performed on Family 8 as parental samples were not available. Genomic DNA was captured using the SureSelect Human All Exon V5 kit (Agilent Technologies, Santa Clara, CA, USA) and sequenced on an Illumina HiSeq 2000 or HiSeq 2500 (Illumina, San Diego, CA, USA) with 101-bp paired-end reads.<sup>19</sup> Image analysis and base calling were performed using sequence control software with real-time analysis and CASAVA software (Illumina). Reads were aligned to GRCh37 using Novoalign (<http://www.novocraft.com/>). Marking of PCR duplicates, indel realignment, and base quality score recalibrations were performed using Picard (<http://picard.sourceforge.net/>) and Genome Analysis ToolKit (GATK; <https://www.broadinstitute.org/gatk/index.php>). Variants were called by the GATK UnifiedGenotyper (<http://www.broadinstitute.org/gatk/>) and annotated using ANNOVAR (<http://www.openbioinformatics.org/annovar/>) after excluding common variants registered in the common dbSNP135 database (minor allele frequency  $\geq 0.01$ ). For Family 7, exome mean depth of coverage for the trio was 87.6 $\times$ , and 95.8% of the total coding sequence of RefSeq genes was covered with a depth of 10 $\times$  reads or more. For proband from Family 8, exome mean depth of coverage was 56.6 $\times$ , and 93.8% of the total coding sequence of RefSeq genes was covered with a depth of 10 $\times$  reads or more.

### **Family and clan structure and identity-by-descent (IBD)**

Absence-of-Heterozygosity (AOH) was calculated in Family 5 and Family 6 from unphased exome data as a surrogate measure of Runs-of-Homozygosity (ROH). This was done using a

bioinformatic tool called BafCalculator (<https://github.com/BCM-Lupskilab/BafCalculator>) that uses a 1.5 Mb cutoff value to call AOH. We calculated the fraction of the genome covered by ROH region as an estimation of the inbreeding coefficient FROH (Fig. S2). In comparison, the expected coefficient of inbreeding FPED was calculated based on the pedigree structure. The AOH in Family 1 was calculated through an in-house custom made script whereas >7 consecutive homozygous single nucleotide polymorphism (SNPs) are considered an AOH region.<sup>11</sup>

### **Exonic copy number variant (CNV) and mutational mechanism**

To characterize this exonic CNV event, determine breakpoint junctions and infer mutational mechanisms from mutation signatures, long-range PCR was designed with a set of primers flanking the deleted region. AluAluCNVPredictor (<http://alualucnvpredictor.research.bcm.edu:3838/>), an in-house developed bioinformatic tool to identify the directly oriented CNV-predicted *Alu* pair intersecting the two genomic intervals, was used to further explore the hypothesized mutational mechanism underlying the exonic deletion and examine the AAMR (*Alu-Alu* Mediated Rearrangement) genomic instability index for *LGI3*.<sup>20</sup>

### **Deep clinical characterization**

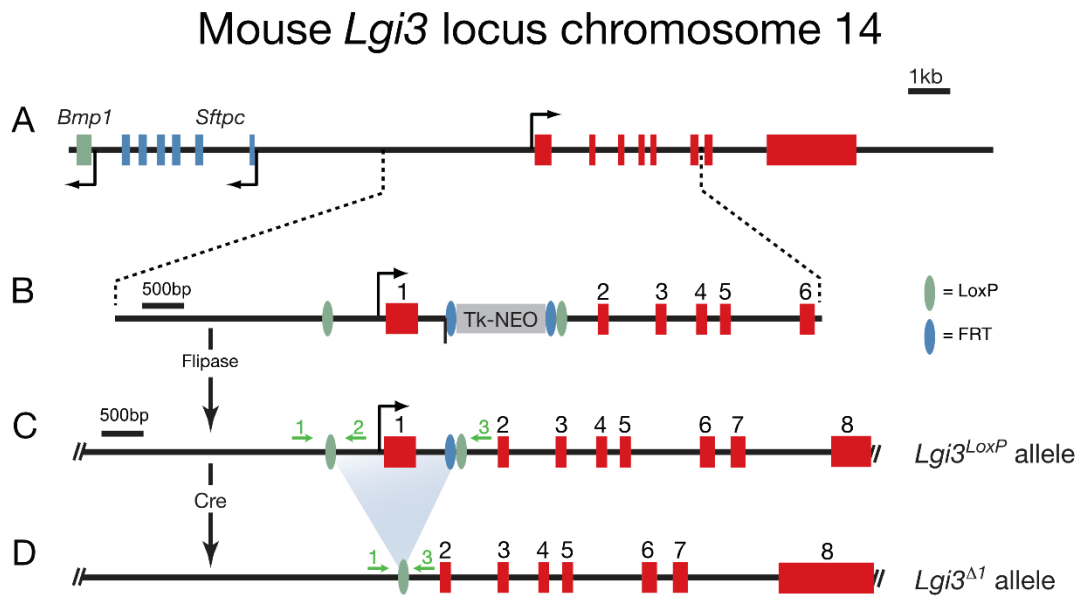
We performed a comprehensive retrospective subjects' medical history and clinical data analysis of the affected subjects including the developmental history, age of onset of disease, growth parameters, central and peripheral neurological features such as tone, reflexes, muscle power, and gait in addition to the presence or absence of abnormal movements such as myokymia and seizures. We also reviewed the findings of the electrodiagnostic and neurophysiological studies including nerve conduction studies (NCS), electromyograms (EMG), electroencephalograms (EEG) in addition to the radiological images, particularly brain Magnetic Resonance Imaging (MRI).



## Mouse genetics and Immunohistochemistry

### Mice genetics

C57BL6/J mice of both sexes were used in this project. The animals were bred and maintained at the Little France animal unit at the University of Edinburgh, Edinburgh UK. The *Lgi3*<sup>Δ1/Δ1</sup> genotype was generated using a standardised gene recombination approach, as previously described.<sup>21</sup> Initially, a LoxP site was inserted upstream of the first exon whilst an Frt-flanked neo cassette with a 3' LoxP site was inserted downstream, resulting in the *Lgi3*<sup>LoxP</sup> allele.<sup>21</sup> The *Lgi3*<sup>Δ1</sup> null allele was then generated through crossing *Lgi3*<sup>LoxP/+</sup> mice with mice carrying germline Cre recombinase leading to deletion of the first exon and its promoter (Figure S1). The *Lgi3*<sup>Δ1/+</sup> mice were eventually intercrossed, generating *Lgi3*<sup>Δ1/Δ1</sup> offspring. *Lgi3*<sup>Δ1/Δ1</sup> mice have a normal life span, are fertile and exhibit no overt abnormal behavior. In line with these findings, another *Lgi3* knock out mouse line analyzed by the International Mouse Phenotyping Consortium (IMPC), confirmed that *Lgi3* null mice are fertile, have a normal lifespan and show no behavioral abnormalities. Genotypes were determined through Polymerase Chain Reaction (PCR) on DNA from ear biopsies. *Lgi3*<sup>LoxP</sup> and WT alleles were genotyped using primers 5'-GCACGCTGTGTTCCCTCAGCTG-3' and 5'-GGGAGAGCAGCCAAAGGATG-3' with expected bands of 302-bp for the WT allele and 344-bp for the floxed allele. The first primer, in combination with primer 5'-GGCAGGAGTCTGGTCCATGC -3' were used for the *Lgi3*<sup>Δ1</sup> allele with expected band of 262-bp for the *Lgi3*<sup>Δ1</sup> allele and 1204-bp for the WT allele. All animal care and procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 (ASPAs).



**Figure S6:** Depiction of the WT mouse *Lgi3* gene locus (mouse chromosome 8) and strategy to generate the *Lgi3<sup>LoxP</sup>* and *Lgi3<sup>Δ1</sup>* alleles.

### Sciatic nerve crush

Sciatic nerves crush surgery was performed in 12 weeks old WT and *Lgi3<sup>Δ1/Δ1</sup>* mice. All surgical instruments were sterilized by autoclaving before the surgery and again between animals using a bead sterilizer. Following a subcutaneous injection with 10% Vetergesic analgesic diluted in saline (0.01mL per 10g bodyweight), the mouse was placed in an anesthesia chamber filled with 4% isoflurane. The flow was reduced to 2% once full anesthesia was achieved. The mouse was removed from the chamber and the fur was clipped from the operated area. Isoflurane-transferring cone was placed on the animal's nose to ensure continuous anesthesia during the procedure. Body temperature, heart rate and respiration were monitored throughout the operation. The skin was disinfected using iodine-soaked sterile cotton swabs, and surgical drapes were placed around the

incision area. Using dissection spring scissors, a vertical incision was made above the right hip. Once exposed, the sciatic nerve was crushed at the sciatic notch with surgical forceps at three rotation angles, each for 15 seconds. The incision was closed with 2 wound clips per mouse. The animal was transferred to a clean cage placed on top of a heat mat and monitored until conscious. Mice were monitored daily for 72 hours after the procedure; wound clips were removed after 10 days. Mice were culled at appropriate post-surgery time points as described below. Left (control) and right (injured) sciatic nerves were harvested and stored separately prior to processing for immunohistochemistry analysis.

### **Immunohistochemistry on mouse sciatic nerve**

Eight weeks old *Lgi3<sup>Δ1/Δ1</sup>* and WT mice were anesthetized using a lethal dose of Sodium Pentobarbital and trans-cardially perfused with 0.1M NaPO<sub>4</sub>, pH7.4, followed by 4% methanol-free paraformaldehyde (PFA) in 0.1M NaPO<sub>4</sub>. Sciatic nerves were isolated and post-fixed in 4% PFA solution for 20min. Tissue was then transferred to 0.1M NaPO<sub>4</sub> buffer and stored at 4°C until further processing (no longer than 48 hours). To obtain single axon fibres, nerves were initially transferred onto Superfrost Plus™ adhesion microscope slides (ThermoFisher-Scientific) with a large drop of phosphate buffer and cut into shorter fragments. Epineural membrane was removed, and nerve bundles were gently teased apart into single axons using extended acupuncture needles. Teased nerves on slides were air-dried at room temperature (RT) overnight and either used immediately or frozen at -20°C until further use. Before staining, slides with dried or frozen teased nerves were rehydrated with three 5-minute washes in Phosphate Buffered Saline (PBS) followed by a 10-minute incubation in ice-cold 100% Methanol at -20°C. Methanol was washed off with another three 5-minute PBS washes. The slides were transferred into a humidified box and incubated for 1 hour at RT with fish skin gelatin blocking solution (0.3% Triton-X100 in

Sørensen's phosphate buffer, 5% fish skin gelatin - FSG (Sigma-Aldrich)). Primary antibodies were diluted in the same blocking buffer and applied to slides (Table 1.5A). Incubation with primary antibodies was done in a humidified box at 4°C overnight. Slides were then washed six times for 15 minutes at RT with Tris-Buffered Saline (TBS)+ 0.01% Triton-X100. Secondary antibodies (Table 1.5B) were diluted in blocking buffer and incubated on slides for 1 hour in a humidified, light-tight box at RT. This was followed by six 15-minute washes with TBS/0.01% Triton-X 100, one with TBS and one with demi-water. Slides were air-dried in a light-tight box at RT and cover slips were mounted with Mowiol medium. Imaging was done using Zeiss Axio Imager Z1 ApoTome microscope (Carl Zeiss). WT and KO nerve slides were subjected to the same exposure times for reliable analysis. All immunofluorescence images were processed and analysed using Fiji software (ImageJ). Figures were composed using Affinity Designer software.

Table S3

Target	Isotype	Dilution	Producer	RRID
CASPR (CNTNAP1)	mouse IgG1	1/200	Lab of Elior Peles	AB_2314220
CASPR (CNTNAP1)	rabbit	1/500	Abcam (ab34151)	AB_869934
Kv1.1 (KCNA1)	rabbit	1/200	Meijer lab UoE	
Kv1.2 (KCNA2)	mouse IgG2b	1/25	Neuromab (K14/16)	AB_2877295
LGI3	rabbit	1/200	Proteintech (21919-1-AP)	AB_10838807
CASPR2 (CNTNAP2)	rabbit	1/500	Genscript (A01426-100)	AB_10838807
PSD95 (DLG4)	mouse IgG2a	1/20	Neuromab (K28/43)	AB_2750659
ADAM23	chicken	1/100	Meijer lab UoE	

Table S4

Source and target	Fluorophore	Dilution	Company	RRID
Goat anti-Mouse IgG1	Alexa488	1/400	ThermoFisher (A21121)	AB_2535764
Donkey anti-Rabbit IgG	Alexa488	1/400	Jackson ImmunoResearch (711-545-152)	AB_2313584
Goat anti-Mouse IgG2a	Alexa555	1/400	ThermoFisher (A21137)	AB_2535776
Goat anti-Mouse IgG2b	Alexa555	1/400	ThermoFisher (A21147)	AB_2535783
Donkey anti-Rabbit	Alexa555	1/400	ThermoFisher (A31572)	AB_162543
Donkey anti-chicken	Alexa647	1/400	Jackson ImmunoResearch (703-605-155)	AB_2340379

### Cell culture

HEK293T cells were grown in DMEM supplemented with 10% FBS and 5000 units/ml of Penicillin and Streptomycin at 37°C in a 5% CO<sub>2</sub> incubator. Cells were transfected using PEI-Max or Lipofectamine 3000 following established protocols. Ni-NTA agarose precipitation, Western blotting and quantification was performed as described previously.<sup>22</sup>

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