Supplementary material

1. Cohorts screened by next generation sequencing

1. *Cohort from Wolfson Medical Center, Holon, Israel*

   This cohort includes 15 patients, 8 female and 7 male, with various forms of epilepsy from Israel. All but one, have intellectual disability with various degrees. One case has a sibling with the same clinical findings consisting of ID, microcephaly and epilepsy. In one case X-linked inheritance is suspected. Five cases are diagnosed with epileptic encephalopathy (EE). Three cases have MRI changes. Four cases have microcephaly. Whole exome sequencing revealed no mutations in the *GABRA3* gene.

2. *Cohort from the European MRX consortium and associated groups (X-chromosome exome sequencing)*

   This cohort includes 480 index cases from families with suggestive syndromic or non-syndromic X-linked intellectual disability that remained unresolved by pre-screening for mutations in selected known XLID genes and by array CGH. All index cases had a normal karyotype and were negative for *FMR1* repeat expansion. Each of the families has at least 2 affected males and in the majority of the families affected males were present in separate sibships. Index cases have been studied using genome partitioning and NGS as previously described (Hu *et al.*, 2014; Hu *et al.*, 2016).

3. *Cohort of 600 cases undergoing diagnostic high-density array-CGH screening*

   Clinical records of patients with unexplained epilepsy (n° = 198, 178 males), epilepsy and intellectual disability (n° = 103, 75 males) or intellectual disability without epilepsy (n° = 299, 125 males) undergoing array-CGH screening as routine diagnostic procedure were prospectively collected. Clinical evaluation included neurological examination, EEG recordings, brain magnetic resonance imaging and drug history. Seizures and type of epilepsy were defined according to classification of epilepsies and epileptic syndromes of the International League against Epilepsy (1989). IQs were defined according to *Diagnostic and Statistical Manual of Mental Disorders*: borderline intellectual disability, IQ 71-84; mild intellectual

disability, IQ 60-70; moderate intellectual disability, IQ 50-59; severe intellectual disability, IQ < 50). Patients with lesional or metabolic cause of epilepsy, major congenital malformations, and major neuropsychiatric diseases (eg. schizophrenia, autistic spectrum disorder) have been excluded.

4. *Cohort IGE/GGE families from EuroEPINOMICS (whole exome sequencing)*

Cohort description (www.esf.org/euroepinomics). The cohort consists of 238 independent individuals with mainly idiopathic/genetic generalized epilepsy (IGE/GGE) including the clinical subsyndromes childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE) and juvenile myoclonic epilepsy (JME) in combination with generalized spike-wave epileptic discharges (GSW) on electroencephalograms (EEG). The patients were recruited in Finland, France, Germany, Italy, The Netherlands and Turkey. All individuals were whole exome sequenced within the Euroepinomics CoGIE-IGE consortium. The index cases with CAE, JAE or JME were chosen from families with at least one additional case of IGE/GGE or generalized spike-wave discharges on EEG. However, ten sporadic cases and 27 patients with generalized-tonic clonic seizures (GTCS) only and GSW on EEG were also included, when they were part of families with mainly CAE, JAE or JME phenotypes and not enough DNA was available from those other family members. Combinations of more than one subsyndrome within one individual occurred as well. The cohort thus consists of the following groups: 118 cases with CAE, 29 JAE, 50 JME, 7 CAE/JME, 7 JAE/JME, and 27 cases with GTCS only.

2. Patient phenotypes

Family 1

This family includes the parents and their four children. The father is healthy with no neurological diseases in his family. The mother (**patient II:2**) is 42 years old. She was born with cleft palate and nystagmus and had developmental delay and learning disabilities. She was operated because of scoliosis. From 21 years of age, she experienced four isolated generalized tonic-clonic seizures. Her EEG showed generalized slowing of the background activity. She was treated with carbamazepine during all of her pregnancies. One seizure recurred on attempt to discontinue treatment. Her height is 150 cm (3rd centile), with a normal head circumference. She has mild dysmorphic features including microretrognathia and synophris. She has horizontal nystagmus but otherwise normal neurological examination. Brain MRI is normal.

Patient III:1 is 18 years old. He was born after a normal pregnancy with a cleft palate and horizontal nystagmus. At the age of 2 months he presented with infantile spasms and was treated with ACTH, vigabatrin and clonazepam until the age of 14 months. At the age of 3.5 years he started to have frequent falls and atypical absences with developmental regression and was diagnosed with Lennox-Gastaut syndrome. Over the years, he has been treated with many antiepileptic drugs and vagal stimulation but his epilepsy remained intractable. EEG done at the age of 7 months demonstrated parietal spikes. He suffers from severe intellectual disability and lives in an institute for disabled patients. He is currently on levetiracetam, valproic acid and rufinamide. A metabolic evaluation, muscle biopsy and brain MRI were normal. His height is 130 cm (the 10^{th} centile for 11 years), with severe scoliosis and his head circumference is 51 cm – 2^{nd} centile for 11 years. He has micrognathia, a short neck, sloping shoulders and scoliosis. He does not speak at all and there is only short eye contact with horizontal nystagmus. He walks in a crouched gait.

Patient III:2 is a 14 year old girl who was born after a normal pregnancy and delivery. She started walking at the age of 2.5 years and has learning disabilities. At the age of seven years she developed generalized tonic-clonic seizures at awakening. She was treated with lamotrigine with excellent response. During the past year she has had frequent falls with seizure recurrence. She was recently hospitalized for generalized seizures and status epilepticus. Valproic acid was added. Her EEGs showed epileptiform complexes with short bursts of spikes and multi spikes and a generalized slowing. Brain MRI was not contributory. Her height is 149.5 cm (5^{th} percentile) and her head circumference is 54 cm (50^{th} percentile). She has retrognathia and sloping shoulders with mild nasal speech and fine horizontal nystagmus when looking sideways.

The third child **III:3** , an eight year old boy, is normally developed with no epilepsy, no cleft palate and no dysmorphic features.

Patient III:4 was born after 35 weeks gestation weighing 2030 g. A fetal ultrasound demonstrated micrognathia and a short neck. He was born with a cleft palate and demonstrated global developmental delay. He walked at the age of 3 years and spoke in short sentences at 6 years. Epilepsy was diagnosed at 3 years of age and several routine EEGs were reported as normal. Video EEG performed at the age of 8 years revealed clusters of epileptic spasms and tonic seizures. The spasms were asymmetric, involving the right arm and correlated with a slow wave over the left centro-parietal region. The tonic seizures were symmetric involving the arms with tonic posturing of the whole body, correlated with diffuse voltage attenuation superimposed by beta activity. There were polymorphic delta waves and spike-wave complexes over the left frontocentral region. He

was treated with levetiracetam, clobazam, carbamazepine and lamotrigine but never became seizure-free. He was diagnosed as having moderate intellectual disability. He passed away at the age of 10 years after choking on food during a seizure. He had horizontal nystagmus and sloping shoulders and the same dysmorphism as his mother and sister. Head circumference was normal. A brain MRI was normal.

Family 2

Patient II:1 has cognitive dysfunction of unknown cause. Linkage analysis revealed that he carries a different haplotype.

Patient II:2 had generalized epilepsy from 5 till 14 years of age (seizures in the morning) and is not co-operative.

Patient II:4 had an incident of loss of consciousness in adulthood.

Patient II:6 had absences, achieved a very poor degree of education (only basic school), personal contact with her is very difficult. She was pregnant 8 times, she miscarried twice and once she lost a child in the 6th month of pregnancy. She gave birth to 5 children (2 boys and 3 girls). She presented with physical features also present in her two affected sons (III:10 and III:13) – tall, slim stature, dysmorphic features: big, low set ears, micrognathia, long fingers.

Patient III:3 had school difficulties, small mouth, micrognathia.

Patient III:4 – intellectual disability. He graduated from special school for disabled.

Patient III:6 – unaffected. He is currently finishing higher education.

Patient III:7 – mild intellectual disability. He was born from the second gestation. His Apgar score was 8. Psychomotor development of up to 1 year of age was normal (sitting 6/12; walking 12/12). After 8 years of age he had nocturnal enuresis, but the defect of the urinary tract was excluded. He had neither seizures nor loss of consciousness. At the age of 8 years he was subjected to psychological testing because of learning difficulties. Dyslexia and dysortography were diagnosed. Intellectual level was considered as average. He presented with hyperactivity, speech defect and disturbances of visual-motor integration. Speech therapy was prescribed. Last physical examination at the age of 21 years – slim physique, height

170 cm, elongated skull, long neck, sharply ended and long nose, no nystagmus, narrow and narrowly spaced palpebral fissures, arched palate, large protruding ears, sloping shoulders, long fingers, second and third toes – small syndactyly. The patient achieved a poor degree of education and he works physically.

Patient III:10 was born two weeks before delivery term. His birth weight was 2650 g and the birth length was 52 cm. Occipital frontal circumference (OFC) is unknown, 9 Apgar points. He had a delayed speech and spoke his first words at 3 years. At the age of 17 years, he had an attack of unconsciousness and EEG indicated generalized spike and waves complexes. However, he has not been administered any medication. He has a mild intellectual disability. He has a high stature (190 cm in the last examination at the age of 23 years) and several dysmorphic features, including an elongated skull, big, low set ears, micrognathia, arched palate, small mouth, long fingers, broad chest, tall and slim stature, and horizontal nystagmus. Karyotype analysis was normal and Fragile X syndrome was excluded. He is 23 years old now and he graduated from special school for disabled.

Patient III:12 - school difficulties, dysmorphic features (small mouth, micrognathia).

Patient III:13 was born at term. His birth weight was 3270 g and his birth length 57 cm, OFC is unknown, 9 Apgar points. He had speech delay and spoke his first words at 3 years of age. At the age of 12 years he had attacks of unconsciousness during wakefulness. Epilepsy has been diagnosed (EEG indicated generalized spike and wave complexes). He has been treated with valproate for 6 years. He has mild intellectual disability. He has high stature (182 cm on last examination at the age of 21 years) and several dysmorphic features (elongated skull, big, low set ears, micrognathia, arched palate, small mouth, long fingers, broad chest, high, slim stature) and horizontal nystagmus. He is 21 years old now and he graduated from special school for disabled.

Family 3

Patient II:1 is a 16-year-old girl born at term after an uneventful pregnancy. She had a normal neuropsychological development. At the age of 10 months she had a febrile seizure lasting for 10 minutes. The EEG performed the day after the seizure was normal and she was not treated. She never experienced other episodes.

Patient II:2 is a 9-year-old boy born at term after an uneventful pregnancy. No delivery problems were reported. Motor and cognitive development was normal. The patient presented with a simple febrile seizure at the age of 3 years and 4 months. The EEG was normal and he was not treated. Six months later he started to have afebrile generalized tonic-clonic seizures mainly during sleep. Brain MRI, laboratory and metabolic tests were normal. The EEG showed generalized spike- and poli-spike-and-wave complexes, mainly during sleep, and valproate was started. Under this treatment, tonic-clonic seizures reduced in frequency but myoclonic seizures, involving the head and/or upper limbs, and absences with atonia appeared almost daily. Therefore levetiracetam, clonazepam, clobazam, etosuccimide and phenobarbital were tried as add-on treatment to valproate with poor efficacy. At the age of 7 years, he started to present weekly tonic seizures mainly during sleep. Currently he is taking valproic acid 600 mg/day, ethosuximide 600 mg/day, felbamate 900 mg/day and clobazam 10 mg/day (weight: 32 kg). Neuropsychological evaluation, performed at the age of 7.5 years, using Leiter International Performance Scale Revised (Leiter-R) revealed a QIT of 77.

Family 4

Patient II:1 is a now 12-year old girl. Her motor development was unremarkable; she crawled around 8 months and walked independently at 14-15 months. Her speech was difficult to understand, and improved with speech therapy at age 4-5 years. Her first seizure occurred at her third birthday. She has had a variety of seizure types, including partial complex seizures, with tonic and clonic features. The seizures mostly occur in clusters, but occur at least 3-4 times per week. Neurospychological testing at age 5 years showed total IQ of 72 (with VIQ 80 and PIQ 68). Brain MRI (age 7) showed no abnormalities. She was treated with valproic acid, levetiracetam, carbamazepine, and clobazam with much effect. Generalized attacks responded to diphantoine. These attacks were characterized by prodromal features, followed by loss of consciousness, stiffening of arms and legs, screaming and blinking of the eyelids. She has smaller attacks with gagging and eye blinking which can continue for hours, and occur at night. These respond to lorazepam. At age 9 anti-epileptic therapy was seized for diagnostic evaluations. She developed a status epilepticus with a

continuous cluster of seizures. Since then she is on oxcarbazepine but has had daily attacks since. Recently she developed “screaming attacks”, early in the morning she becomes very frightened, screams, and the attack is over. Fear preceded her attacks. She developed generalized fear, was afraid to walk. Since a few months she is on fluoxetine therapy, which has given a great reduction of the anxiety.
EEG recording did not show a conclusive focus, however there is a suspicion of a frontal/central/parasagittal focus.

Family 5

The family has two healthy sons and one affected daughter.

Patient II:1 Development seemed normal in the first year, although slower than siblings, walking at age 18 months. Speech development was delayed. At birth a microtia on the right was noted, and later severe unilateral hearing loss on that side. Strabismus OS, surgically corrected at age 4 years, normal vision. First epileptic seizures at age two years, complex partial seizures with tonic component. On EEG a central focus. In retrospect probably absence like episodes in the first year of life. She is drug resistant. Moderate reduction of seizure frequency after implantation of a neurovalgus stimulator and later ketogenic diet. Her behavior shows autism like features. IQ measurements 65-70, recently ~50. Height –2 SD, otherwise healthy.

Family 6

This family has two affected males and one healthy sister. They were born from consanguineous parents and they have another healthy half-brother. Both affected boys were born after a normal pregnancy.

Patient II:1 started walking at 12 months and spoke his first words at around 6 years. At 12 years he could make simple sentences and write his name. At 17 years he could speak in sentences, read and use public transportation. He presented with autism spectrum disorder associated with obsessive-compulsive behavior, agitation and phobias. He also had sleeping problems. There was no hint for epileptic seizures.

Patient II:2 started walking at 9 months and, similar to his brother, had behavioural disturbances, such as agitation, aggressiveness, stereotypies and hypersensitivity to noise. He also had sleeping problems. No hints for epileptic seizures.

Their half-brother had a delayed language development. At 4 years he could speak simple sentences. He attended a regular school but had learning difficulties.

Family 7

This Italian family comprises three individuals affected by IGE/GGE. All affected members had a normal neurological examination and no hint for dysmorphisms or intellectual disability.

Patient I:1 suffered from a few tonic-clonic seizures at random from 10 to 12 years old, suggestive of EGTCS only. His EEG showed generalized spike-and-wave complexes. He was treated for several years with valproate and is currently seizure-free without therapy.

Patient II:1 is a 17-year-old girl who suffered from age 7 years on from daily, frequent (up to hundreds each day) episodes of sudden, brief impairment of consciousness and interruption of the ongoing activity (classified as pyknoleptic absence seizures). She also had occasional tonic-clonic seizures at age 8 years. Her EEGs showed regular and symmetrical generalized discharges of 3-3.5 Hz spike and wave complexes on normal background activity, as typically observed in childhood absence epilepsy (CAE). She was successfully treated with valproate (500 mg/day). Seizures remitted within 5 years from onset and the patient is currently without medication.

Patient II:2 is a 15-year-old girl who experienced four generalized tonic-clonic seizures (GTCS) precipitated by sleep deprivation in adolescence, suggestive of epilepsy with GTCS only (EGTCS). Her EEGs showed generalized discharges of 3.5–4 Hz spike and wave complexes on normal background activity. She did never receive anti-seizure treatment and is currently seizure-free.

Supplementary Table 1. Scores from prediction softwares

Family	Mutation	PolyPhen-2	SIFT	MutationTaster	ExAC	gnomAD
1	p.Q242L	0.985 (probably damaging)	0.11 (tolerated)	0.999998345510216 (disease causing)	0	0
2	p.T166M	1.000 (probably damaging)	0.19 (tolerated)	0.999980339497156 (disease causing)	0	$5.6x10^{-6}$
4, 5	p.Y474C	0.999 (probably damaging)	0 (damaging)	0.99999745898801 (disease causing)	0	0
6	p.G47R	1.000 (probably damaging)	0.04 (damaging)	0.76938630907083 (disease causing)	$1.177x10^{-5}$	$1.163x10^{-5}$
7	p.T336M	1.000 (probably damaging)	0.12 (tolerated)	0.999999448138709 (disease causing)	0	

Supplementary Table 2. List of autosomal dominant variants identified by WES in Family 1

Genomic Position[#]	Gene	Transcript ID	cDNA	Protein	Prediction	MAF (ExAC)	OMIM ID (Disease)	OMIM Description (Disease)	Genetic Model
chr1:31838756	FABP3	NM_004102	c.C379T	p.R127C	PP2 - probably damaging 0.876 MT- disease causing 1 SIFT - deleterious 0.024	0.000008303			
chr1:36824360	STK40	NM_001282546	c.C191T	p.T64M	PP2 - damaging 1.0 MT - disease causing 1 SIFT - deleterious 0.001	0.00003296			
chr3:49742591	RNF123	NM_022064	c.G2134A	p.V712M	PP2 - probably damaging 0.776 MT - disease causing 0.985 SIFT - tolerated 0.219	0.0005			
chr3:53914082	ACTR8	NM_022899	c.A178T	p.I60F	PP2 - damaging 0.962 MT - disease causing 1 SIFT - deleterious	0.000008243			

					0.022				
chr4:155467345	PLRG1	NM_001201564	c.A307G	p.T103A	PP2 – damaging 0.957 MT - disease causing 1 SIFT – tolerated 0.451	NA			
chr4:155468976	PLRG1	NM_001201564	c.G146T	p.R49L	PP2 - benign 0.003 MT - disease causing 1 SIFT - tolerated 0.221	NA			
chr5:100191849	ST8SIA4	NM_005668	c.G755A	p.R252Q	PP2 - benign 0.195 MT - disease causing 1 SIFT - deleterious 0.006	0.00001648			
chr5:127420178	SLC12A2	NM_001046	c.G532C	p.V178L	PP2 - benign 0.091 MT - disease causing 1 SIFT - tolerated 0.24	NA			
chr5:176011668	CDHR2	NM_001171976	c.G2386A	p.V796M	PP2 - damaging 1.0 MT - disease causing 1 SIFT - deleterious 0.009	0.00009929			
chr5:176562294	NSD1	NM_022455	c.G190A	p.D64N	PP2 - probably damaging 0.713 MT - disease causing 1	NA	130650 601626 117550	Beckwith-Wiedemann syndrome	AD AD AD

					SIFT - deleterious 0.005			Leukemia, acute myeloid Sotos syndrome 1	
chr6:109787476	ZBTB24	NM_014797	c.G1672A	p.D558N	PP2 - benign 0.033 MT - disease causing 0.974 SIFT - tolerated 0.163	0.0025	614069	Immunodeficiency-centromeric instability-facial anomalies syndrome-2	AR
chr6: 130762619	TMEM200A	NM_001258276	c.T1052C	p.I351T	PP2 - benign 0 MT - neutral 0.999 SIFT - tolerated 0.226	0.0038			
chr8:25149646	DOCK5	NM_024940	c.A428G	p.E143G	PP2 - damaging 0.997 MT - disease causing 1 SIFT - deleterious 0.003	NA			
chr9:35092752	PIGO	NM_032634	c.C1132T	p.L378F, PIGO	PP2 - damaging 1.0 MT - disease causing 1	0.0001	614749	Hyperphosphatasia with	AR

					SIFT - deleterious 0.007			mental retardation syndrome 2	
chr10:123845115	TACC2	NM_001291876	c.G3100A	p.E1034K	PP2 - probably damaging 0.885 MT - disease causing 1 SIFT - deleterious 0	0.0033			
chr10:134039067	STK32C	NM_173575	c.C736T	p.R246W	PP2 - damaging 1.0 MT - disease causing 0.999 SIFT - tolerated 0.177	0.0003			
chr11:102201850	BIRC3	NM_001165	c.G1202A	p.R401K	PP2 - benign 0.0 MT - neutral 0.962 SIFT - tolerated 0.152	0.0059			
chr16:921293	LMF1	NM_022773	c.A946G	p.M316V	PP2 - benign 0.002 MT - disease causing 0.942 SIFT - tolerated 0.069	0.000008732	246650	Lipase deficiency, combined	AR
chr16:3254964	OR1F1	NM_012360	c.A718G	p.T240A	PP2 - benign 0.208 MT - disease causing 0.997	0.0005			

					SIFT - deleterious 0.001				
chr16:3639699	SLX4	NM_032444	c.C3940A	p.Q1314K	PP2 - damaging 0.958 MT - neutral 1 SIFT - tolerated 0.136	0.0003	613951	Fanconi anemia, complementation group P	AR
chr16:67859955	TSNAXIP1	NM_001288994	c.C82T	p.R28W	PP2 - damaging 1.0 MT - neutral 0.929 SIFT - deleterious 0.0	0.0011			
chr17: 1377903	MYO1C	NM_001080779 NM_001080950 NM_033375	c.1797+1G>T c.1740+1G>T c.1692+1G>T	NA	PP2 - MT - disease causing 1 SIFT -	NA			
chr17:8168319	PFAS	NM_012393	c.T2156C	p.I719T	PP2 - benign 0.004 MT – neutral 0.972 SIFT – tolerated 1.0	0.0001			
chr19:18284701	IFI30	NM_006332	c.51delG	p.L17fs	PP2 - MT -	0.001			

					SIFT -				
chr19: 35512773	GRAMD1A	NM_001136199 NM_020895	c.1732+5G>A c.1753+5G>A		PP2 - MT - SIFT-	0.0001			
chr22:42321451	TNFRSF13C	NM_052945	c.C475T	p.H159Y	PP2 - damaging 0.994 MT - disease causing 0.977 SIFT - deleterious 0.0	0.0056	613494	Immunodeficiency, common variable, 4	AR
chr22:50647086	SELO	NA	NA	NA	PP2 - probably damaging 0.903 MT - disease causing 1 SIFT - tolerated 0.056	0.0026			
chrX: 48457853	WDR13	NM_017883 NM_001166426	c.392+3A>G c.116+3A>G		PP2 - MT - SIFT -	NA			
chrX:151376526	GABRA3	NM_000808	c.A725T	p.Q242L	PP2 - damaging 0.985 MT - disease causing 1 SIFT - deleterious 0.0	NA			
chrX:15328201	IRAK1	NM_00102524	c.G1106	p.G369E	PP2 – damaging 0.984	0.0002			

8		2	A		MT – neutral 1 SIFT – tolerated 0.086				

[#]The analysis of family 1 revealed further variants in genes additional to *GABRA3*. Two of those genes, namely *SLC12A2* and *PIGO* might have an influence on the phenotype. For the others, we do not see a plausible explanation to contribute significantly to the phenotype, but of course we cannot exclude minor contributions.

The gene *SLC12A2* ecodes for a $Na^+/K^+/Cl^-$-Symporter (NKCC1). In a Na^+ dependent process, SLC12A2 transports ions (Na^+, K^+, Cl^-) across the cell membrane to maintain intracellular K^+ and Cl^- homeostasis (Cong D et al. 2015). Furthermore, SLC12A2 and KCC2, the Cl^- extruding K^+-$Cl^—$ cotransporter, play an important role during development when the response to GABA is changed from excitatory to inhibitory. This change is mediated by a change of the expression of *SLC12A2* and *KCC2*. SLC12A2 expression peaks at postnatal days 31-41. At that time point KCC2 expression is very low. Later in development, the expression of KCC2 increases while SLC12A2 decreases (Dzhala et al., 2005). As seizures in family 1 are described as infantile or generalized tonic-clonic with an onset from 3 to 21 years, when SLC12A2 expression is expected to be low, it is possible that a variant in *SLC12A2* has only a minor or at best a modifying effect.

The gene *PIGO* is a transferase that is involved in glycosylphosphatidylinositol-anchor biosynthesis (Uniprot: Q8TEQ8). A study by Krawitz P.M and colleagues in 2012 identified compound heterozygous mutations in *PIGO* to be the cause of Hyperphosphatasia with mental retardation syndrome 2 (HPRMS2; OMIM: 614749). HPMRS2 is characterized by intellectual disability with facial dysmorphism, seizures, brachytelephalangy, and persistent elevated serum alkaline phosphatase (hyperphosphatasia) with an autosomal recessive inheritance (Krawitz et al., 2012). In contrast, the *PIGO* variant identified in our study follows an autosomal dominant pattern.

Referring to the ExAC Browser, both variants have a known allele frequency (for the variant in *SLC12A2* it is 0.000009031, for the variant in *PIGO* it is 0.0001383). The variants in *SLC12A2* and *PIGO* are exclusively identified in Family 1 and no further segregating variants in those genes were identified in the other families described within this study. Thus, the *GABRA3* variant is by far the most probable exonic alteration causing the neuropsychiatric disease with epilepsy as a main symptom in this family.

Supplementary Table 3. List of non-synonymous coding variants identified by WES in Family 2

Genomic Position[#]	Gene	Transcript ID	cDNA	Protein	Prediction	MAF (ExAC)	OMIM ID (Disease)	OMIM Description (Disease)	Genetic Model	Shared by*
Chr3:100170600	LNP1	NM_001085451	c.194_195insTCCTAGAAGGCATTCTCATGAGGACCAGGAATTCCGATGCCGATCGTCTGACCGTCT	p.H65delinsHPRRHSHEDQEFRCRSSDRL inframe insertion	-	0.0009 %	-	-	-	1, 3, 5
Chr4:90035109	TIGD2	NM_145715	c.G984C	p.Q328H	PP2 - probably damaging 1.00 MT - polymorphism 0.845 SIFT - deleterious 0.00	-	-	-	-	1
Chr6:100896482	SIM1	NM_005068	c.C616A	p.Q206K	PP2 - probably damaging 0.97	0.0017 %	601665	Obesity, severe	AD,AR	1,5

					MT - disease causing 0.00 SIFT - deleterious 0.00					
Chr9:129 937040	RALGPS1	NM_001190 728	c.T889A	p.S297T	PP2 - benign 0.097 MT - disease causing 1.00 SIFT - tolerated 0.09	-	-	-	-	1
Chr9:130 911821	LCN2	NM_005564	c.T17C	p.L6P	PP2 - probably damaging 0.99 SIFT - deleterious 0.00	0.14%	-	-	-	1
Chr13:51 825601	FAM124A	NM_001242 312	c.101-3C>T	-	MaxEnt: 1.8% NNSPLICE: 0.4% HSF: -8.7%	0.68%	-	-	-	1
Chr13:99 114024	STK24	NM_001032 296	c.1053+4C>A	-	MaxEnt: +2.3% NNSPLICE: +0.0% HSF: +9.8%	0.13%	-	-	-	1,3
ChrX:153 041463	PLXNB3	NM_005393	c.G4523C	p.R1508P	PP2 - probably damaging 1.00	-	-	-	-	2

					MT - disease causing 1.00 SIFT - tolerated 0.29					
ChrX:70145757	SLC7A3	NM_001048164	c.G1766C	p.S589T	PP2 - possibly damaging 0.94 MT - polymorphism 0.00 SIFT - tolerated 0.76	0.23%, 88 hemizygotes	-	-	-	1
ChrX:71521800	CITED1	NM_001144886	c.G355C	p.G119R	PP2 - possibly damaging 0.76 MT - polymorphism 1.00 SIFT - tolerated 0.09	0.15%	-	-	-	1
ChrX:76856021	ATRX	NM_000489.3	c.A5579G	p.N1860S	PP2 - benign 0.00 MT - polymorphism 0.999 SIFT - tolerated 0.77	0.63%, 227 hemizygotes	301040 309580	Alpha-thalassemia/mental retardation syndrome Mental retardation-hypotonic	XLD XLR	1

								facies syndrome, X-linked		

List includes non-synonymous coding variants (MAF < 0.001) shared in a following pattern:

1) III:7+III:10+III:13 shared, not present in III:6
2) III.3+III.6 + III.7+III.10+III.12+III.13 shared, not shared by II.1
3) III:7+III:10+III:13+II:1 shared, not present in III:6
4) III:7+III:10+III:13+II:1+III:12+III:3, not present in III:6
5) III:3+III:7+III:10+III:12+III:13 shared, not present in III:6 and II:1 disregarded

[#]Whole exome sequencing and data filtering resulted in ten additional missense variants present in autosomal and X-chromosomal genes and two autosomal variants which could affect splicing. Of these, all variants except for the missense change in the *PLXNB3* gene, which is located close to *GABRA3* on Xq28, were present in the three affected males III:7+III:10+III:13 and not in the unaffected brother III:6 (indicated by "1" in the last column). However, none of these variants except for the missense change identified in the *SIM1* gene was present in the mildly affected female siblings investigated by WES (III:3, III:12). *SIM1* is not expressed in brain and therefore it is unlikely that the single amino acid exchange identified in this study is responsible for the phenotype. Also, the variants identified in *SLC7A3* and *ATRX* have been reported in a large number of hemizygous normal males in ExAC and are therefore considered to be benign polymorphisms.

None of the other genes has been connected with a Mendelian disease yet.

In summary, we consider it unlikely that any other of the described variants plays a similarly important role for the phenotype in all affected individuals as the *GABRA3* mutation. However, other variants may contribute to or modify the phenotype in certain individuals.

Supplementary Table 4. List of variants identified by WES in Family 3

List of *de novo* Variants									
II:1									
Genomic Position[#]	**Gene**	**Transcript ID**	**cDNA**	**Protein**	**Prediction**	**MAF (ExAC)**	**OMIM ID (Disease)**	**OMIM Description (Disease)**	**Genetic Model**
Chr13:25745730	FAM123A	NM_199138.1	c.28G>T	p.G10C	-	-	-	-	-
II:2									
Genomic Position	**Gene**	**Transcript ID**	**cDNA**	**Protein**	**Prediction**	**MAF (ExAC)**	**OMIM ID (Disease)**	**OMIM Description (Disease)**	**Genetic Model**
Chr8:38814898	PLEKHA2	NM_021623.1	c.831C>A	p.Y277*	-	-	-	-	-
Compound Heterozygous									
II:1									
Genomic Position	**Gene**	**Transcript ID**	**cDNA**	**Protein**	**Prediction**	**MAF (ExAC)**	**OMIM ID (Disease)**	**OMIM Description (Disease)**	**Genetic Model**
Chr16:1388672	BAIAP3	NM_001199096	c.C122T	p.T41M	PP2 – damaging 0.992 MT-neutral 1.00 SIFT – deleterious 0.003	0.0046	-	-	-
Chr16:139118	BAIAP3	NM_001199	c.A524G	p.K175R	PP2 – damaging 1.00	0.0011	-	-	-

5		096			MT-disease causing 1.00 SIFT – deleterious 0.0				
Chr19:11319669	DOCK6	NM_020812	c.T4862C	p.V1621A	PP2 – possibly damaging 0.668 MT- disease causing 1.00 SIFT – deleterious 0.001	0.0022	614219	Adams-Oliver syndrome 2	AR
Chr19:11343895	DOCK6	NM_020812	c.G2702A	p.R901H	PP2 – benign 0.002 MT- disease causing 0.998 SIFT –tolerated 0.122	0.00007	614219	Adams-Oliver syndrome 2	AR
II:2									
Genomic Position	**Gene**	**Transcript ID**	**cDNA**	**Protein**	**Prediction**	**MAF (ExAC)**	**OMIM ID (Disease)**	**OMIM Description (Disease)**	**Genetic Model**
Chr1:183938565	COLGALT2	NM_001303420	c.T670C	p.W224R	PP2 - benign 0.05 MT - disease causing 1.00 SIFT - tolerated 1.0	0.09968	-	-	-
Chr1:183938600	COLGALT2	NM_001303420	c.A635G	p.Y212C	PP2 - probably damaging 1.00 MT- disease causing 1.00 SIFT - deleterious 0.0	0.0021	-	-	-

Chr16: 88787615	PIEZO1	NM_001142864	c.G5627A	p.R1876K	PP2 - benign 0.01 MT - neutral 1.00 SIFT - tolerated 0.39	0.0083	194380 616843	Dehydrated hereditary stomatocytosis with or without pseudohyperkalemia and/or perinatal edema; L ymphedema, hereditary, III	AD,AR
Chr16: 88804731	PIEZO1	NM_001142864	c.C752T	p.A251V	PP2 - benign 0.001 MT - neutral 1.00 SIFT - tolerated 0.226	0.0004	194380 616843	Dehydrated hereditary stomatocytosis with or without pseudohyperkalemia and/or perinatal edema; L ymphedema, hereditary, III	-
Chr18:	ESCO1	NM_052911	c.A466G	p.K156	PP2 - benign 0.002	0.0007	-	-	-

19154339					MT - neutral 1.00 SIFT - deleterious 0.021				
Chr18: 19154417	ESCO1	NM_052911	c.387_388ins TTG:	p.E130delin sLE	PP2 - MT- SIFT -	-	-	-	-

[#]None of the detected variants indicates any potential association with the epileptic or ID phenotype.

Supplementary Table 5. List of variants identified by WES in Family 7

Genomic Position[#]	Gene	Transcript ID	cDNA	Protein	Prediction	MAF (ExAC)	OMIM ID (Disease)	OMIM Description (Disease)	Genetic Model
chr1:26628180	UBXN11	NM_001077262	c.100+5G>A		PP2 - MT- SIFT -	0.0008			
chr1:27223980	GPATCH3	NM_022078	c.C688T	p.R230W	PP2 - 1 MT- disease causing 1 SIFT - deleterious 0.002	0.0001			
chr1:27721192	GPR3	NM_005281	c.891_897del	p.Y297fs	PP2 - MT- SIFT -	NA			
chr1:28282259	SMPDL3B	NM_001009568	c.G755A	p.R252Q	PP2 - damaging 1 MT- disease causing 0.721 SIFT- tolerated 0.143	0.0024			
chr1:36823881	STK40	NM_001282546	c.C316A	p.H106N	PP2 - benign 0.425 MT- disease causing 0.999 SIFT - tolerated 0.126	0.0003			

chr1:85594430	WDR63	NM_001288563	c.C2240G	p.T747R	PP2 - damaging 0.999 MT - disease causing 0.979 SIFT - deleterious 0.004	0.0013			
chr1:94485269	ABCA4	NM_000350	c.T5065C	p.S1689P	PP2 - benign 0.25 MT - disease causing 0.966 SIFT - tolerated 1	0.000009255	604116 248200 248200 601718 248200 153800	Cone-rod dystrophy 3 Fundus flavimaculatus Retinal dystrophy, early-onset severe Retinitis pigmentosa 19 Stargardt disease 1 Macular degeneration, age-related, 2	- AR AR - AR AD
chr2:28742590	PLB1	NM_001170585	c.C203T	p.S68F	PP2 - damaging 0.999 MT - disease causing	0.0002			

					0.634 SIFT - tolerated 0.061				
chr2:39187008	ARHGEF33	NM_001145451	c.G1562C	p.S521T	PP2 - benign 0 MT - neutral SIFT - tolerated 0.281	0.0077			
chr2:55544736	CCDC88A	NM_001135597	c.A3563G	p.K1188R	PP2 - damaging 0.999 MT - disease causing 0.92 SIFT - tolerated 0.721	0.0118	260565	PEHO syndrome	AR
chr2:61415787	USP34	NM_014709	c.C10091A	p.T3364N	PP2 - benign 0.037 MT - neutral 0.539 SIFT - tolerated 0.088	0.00008282			
chr2:136620318	MCM6	NM_005915	c.G1079A	p.G360D	PP2 - damaging 1 MT - disease causing 1 SIFT - deleterious 0	NA	601806	Lactase persistence/nonpersistence	AD
chr2:155252502	GLANT13	NM_052917	c.1157-1G>T		PP2 - MT - disease causing 1 SIFT -	NA			
chr2:172411153	CYBRD1	NM_001256909	c.G503A	p.R168H	PP2 - damaging 1 MT - disease causing 1 SIFT - deleterious 0.001	0.0037			

chr2:190708712	PMS1	NM_001289409	c.G77A	p.R26K	PP2 - benign 0.011 MT - disease causing 1 SIFT - tolerated 0.601	0.012			
chr3:47454394	PTPN23	NM_001304482	c.4253dupC	p.S1418fs	PP2 - MT- SIFT -	NA			
chr4:155530877	FGG	NM_000509	c.G571A	p.G191R	PP2 - damaging 1 MT - disease causing 1 SIFT - deleterious 0.021	0.0027	202400 616004 616004 202400	Afibrinogenemia, congenital Dysfibrinogenemia, congenital Hypodysfibrinogenemia Hypofibrinogenemia, congenital	AR - - AR
chr5:131729380	SLC22A5	NM_003060	c.G1463A	p.R488H	PP2 - damaging 0.984 MT - disease causing 0.91 SIFT - deleterious 0.005	0.0033	212140	Carnitine deficiency, systemic primary	
chr5:1321590	SHROOM	NM_133456	c.G2162T	p.S721I	PP2 - damaging 0.999	0.000009			

06	1				MT- disease causing 0.817 SIFT - deleterious 0.012	299			
chr6:38805800	DNAH8	NM_001206927	c.4449delG	p.E1483fs	PP2 - MT- SIFT -	NA			
chr6:64430522	EYS	NM_001142800	c.T9405A	p.Y3135X	PP2 - MT - disease causing 0.42 SIFT -	NA	602772	Retinitis pigmentosa 25	
chr6:89967489	GABRR2	NM_002043	c.G1298A	p.R433H	PP2 - benign 0.002 MT - disease causing SIFT -	0.0037			
chr6:111587579	KIAA1919	NM_153369	c.815dupC	p.S272fs	PP2 - MT- SIFT -	0.0007			
chr6:116442212	COL10A1	NM_000493	c.G1067A	p.G356D	PP2 - damaging 1 MT - disease causing 1 SIFT - deleterious 0	NA			
chr7:21789835	DNAH11	NM_001277115	c.8798-5G>A		PP2 - MT-	0.0074	611884	Ciliary dyskinesia,	AR

					SIFT -			primary, 7, with or without situs inversus	
chr7:30721569	CRHR2	NM_001202482	c.G191T	p.C64F	PP2 - damaging 1 MT - disease causing 1 SIFT - deleterious 0.001	0.00002996			
chr7:42006036	GLI3	NM_000168	c.G2635A	p.A879T	PP2 - probably damaging 0.876 MT - disease causing 1 SIFT - tolerated 0.084	0.0005	175700 146510 174200 174700 241800	Greig cephalopolysyndactyly syndrome Pallister-Hall syndrome Polydactyly, postaxial, types A1 and B Polydactyly, preaxial, type IV Hypothalamic hamartomas,	AD AD AD AD -

								somatic	
chr7:47342790	TNS3	NM_022748	c.C3215T	p.T1072M	PP2 - damaging 0.987 MT - neutral 1 SIFT - deleterious 0.022	0.0055			
chr8:101717819	PABPC1	NM_002568	c.T1685C	p.L562S	PP2 - damaging 1 MT - disease causing 1 SIFT - deleterious 0	0.0006			
chr8:144733475	ZNF623	NM_014789	c.A1433G	p.Q478R	PP2 - benign 0.159 MT - neutral 0.987 SIFT - tolerated 0.127	NA			
chr9:164194999	BNC2	NM_017637	c.G2788A	p.D930N	PP2 - damaging 1 MT - disease causing 1 SIFT - tolerated 0.308	0.00009073			
chr9:140008443	DPP7	NM_013379	c.C359T	p.S120F	PP2 - damaging 0.997 MT - disease causing 1 SIFT - deleterious 0	0.0135			
chr13:24380110	MIPEP	NM_005932	c.1826delG	p.G609fs	PP2 - MT - SIFT -	0.00008237	617228	Combined oxidative phosphorylation deficiency 31	AR
chr14:682529	ZFYVE26	NM_015346	c.C3025T	p.R1009W	PP2 - damaging 0,999	0.0003	270700	Spastic	AR

45					MT - disease causing 0.859 SIFT - deleterious 0.003			paraplegia 15, autosomal recessive	
chr15:78783019	IREB2	NM_004136	c.G2240A	p.G747E	PP2 - damaging 1 MT - disease causing 1 SIFT - deleterious 0	0.0024			
chr16:597706	CAPN15	NM_005632	c.C868G	p.L290V	PP2 - benign 0.278 MT - neutral 0.996 SIFT - tolerated 0.145	NA			
chr16:1867190	HAGH	NM_001286249	c.G515A	p.W172X	PP2 - MT - disease causing 1 SIFT -	0.0037	614033	Glyoxalase II deficiency	AD
chr16:2353972	ABCA3	NM_001089	c.A1465T	p.M489L	PP2 - benign 0 MT - neutral 1 SIFT - tolerated 0.739	0.0003	610921	Surfactant metabolism dysfunction, pulmonary, 3	AR
chr16:7726818	RBFOX1	NM_145891	c.1036_1037ins CTGCCG	p.T346delins TAA	PP2 - MT - SIFT -	NA			
chr19:1037716	CNN2	NM_201277	c.G630A	p.M210I	PP2 - damaging 0.993 MT - disease causing 1	0.00006856			

					SIFT - deleterious 0.002				
chr19:1037718	CNN2	NM_201277	c.G632T	p.G211V	PP2 - damaging 1 MT - disease causing 1 SIFT - deleterious 0.001	0.00006855			
chr19:8160848	FBN3	NM_032447	c.G5656A	p.D1886N	PP2 - damaging 0.999 MT - disease causing 1 SIFT - deleterious 0	NA			
chr19:8999468	MUC16	NM_024690	c.G40707C	p.W13569C	PP2 - damaging 0.995 MT - disease causing 0.975 SIFT - deleterious 0.017	NA			
chr19:21607223	ZNF493	NM_175910	c.C1378T	p.L460F	PP2 - damaging 1 MT - neutral 1 SIFT - deleterious 0.038	0.0098			
chr19:30498541	URI1	NM_001252641	c.G628C	p.D210H	PP2 - probably damaging 0.93 MT - neutral 0.989 SIFT - deleterious 0.006	NA			
chr19:38189932	ZNF607	NM_001172677	c.G1097C	p.C366S	PP2 - damaging 1 MT - neutral 1 SIFT - deleterious 0.001	NA			

chr19:39227916	CAPN12	NM_144691	c.1241_1242insGC	p.G414fs	PP2 - MT - SIFT -	NA			
chr19:54974460	LENG9	NM_001301782	c.G250T	p.D84Y	PP2 - damaging 1 MT - disease causing 1 SIFT -	0.011			
chr19:55685954	SYT5	NM_001297774	c.C879G	p.I293M	PP2 - probably damaging 0.936 MT - disease causing 0.998 SIFT - deleterious 0.001	0.0004			
chr20:62839452	MYT1	NM_004535	c.904_906del	p.302_302del	PP2 - MT - SIFT -	0.0023			
chr22:30189643	ASCC2	NM_001242906	c.C1483T	p.R495W	PP2 - damaging 0.999 MT - disease causing 1 SIFT - deleterious 0.015	0.0032			
chr22:46929756	CELSR1	NM_014246	c.C3312G	p.F1104L	PP2 - damaging 0.999 MT - disease causing 0.971 SIFT - deleterious 0	0.0003			

chrX:148564708	IDS	NM_000202	c.C1222T	p.P408S	PP2 - probably damaging 0.722 MT - disease causing 1 SIFT - deleterious 0.099	0.000023	309900	Mucopolysaccharidosis II	XLR

[#]The exome sequencing analysis revealed additional variants in family F7 shared by I:1, II:1 and II:2 (MAF < 0.01). From the identified variants, only one variant detected in another subunit of the $GABA_A$ receptor could additionally affect the phenotype in this family. *GABRR2* codes for the ρ2 subunit of the $GABA_A$ receptor. So far, variants in this subunit have not been directly linked to epilepsy, but it was found that polymorphisms in this gene would increase the susceptibility to epilepsy in some populations (Cavalleri et al., 2007; Kumari et al., 2011). Diseases associated with *GABRR2* include Alcohol Dependence and Retinitis Pigmentosa (Marcos et al., 2000; Xuei et al., 2010). Therefore, we may assume that the variant found in this gene may have a contribution to the epileptic disease in the father and the two daughters and may worsen the phenotype of patient II-2 carrying the *GABRA3* variant.

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