The American Journal of Human Genetics, Volume *101*

Supplemental Data

De Novo Variants in *GRIA4* Lead to Intellectual Disability with or without Seizures and Gait Abnormalities

Sonja Martin, Adam Chamberlin, Deepali N. Shinde, Maja Hempel, Tim M. Strom, Allison Schreiber, Jessika Johannsen, Lilian Bomme Ousager, Martin J. Larsen, Lars Kjaersgaard Hansen, Ali Fatemi, Julie S. Cohen, Johannes Lemke, Kristina P. Sørensen, Katherine L. Helbig, Davor Lessel, and Rami Abou Jamra

Supplemental Note: Case Reports

Proband 1

The first proband is a 15-year-old boy, born to healthy, non-consanguineous parents. The pregnancy, achieved by in vitro fertilisation (IVF), was uneventful. He was born at term with a normal birth weight of 3755 g and an APGAR score of 9 and 10. Neonatally he was irritable with trembling and brisk reflexes to noise. In addition, he was unusually stiff, which became more apparent over the next days. Throughout his childhood he has been irritable, anxious and tense with hyperekplexia, tingling and exaggerated head-retraction reflex, with stiffness and hypertonia. After trauma (e.g. from falling) he had up to one-hour bursts of muscle cramps/seizures during which he stayed conscious. EEG at sleep was normal. MRI of cerebrum at one month revealed dilation of the posterior horns of the lateral ventricles but MRI at three years of age was normal. Ophthalmological examination was normal and he has no hearing problems.

He was able to sit at eight months and to walk, with a stiff gait, at 18 months. His development has, however, been otherwise retarded. At age of six years, evaluation showed a developmental stage of a two and a half to three years old child. He has attended special school and is able to write his name and can count. He speaks (with dysarthria) and can run and drive a bicycle. He is social in relation to adults and smiles but clearly annoyed by the hyperekplexia in his interaction with others. He is easily overstimulated and need then rest. He has a reduced attention span. In childhood, he had severe sleeping problems due to spontaneous muscle contractions but this has improved though even small stimuli still triggers muscle contractions. Growth has been normal. At the age of 15 years his height was 175.8 cm and his weight was 74.4 kg. No dysmorphic features are recognized apart from large ears.

Chromosome microarray, testing for Fragile X syndrome, analysis of Thomsen disease (myotonia congenita) and testing for the GLRB, GLRA1, and SLC6A5 genes was normal.

Proband 2

The second proband is a 21-year-old male, born to healthy, non-consanguineous parents. He was born at term following an uncomplicated pregnancy and was delivered by Cesarean section due to fetal heart decelerations. His birth weight was 3320 g. The birth length and head circumference are not known. After birth, he was pale and did not cry immediately and was noted to have jumpy movements with eye blinking, but was discharged after two days. He was very irritable and had episodes of stiffness. He was diagnosed with bilateral hiatal hernias at three weeks and seizures at five weeks. He had reflux, apneas and recurrent respiratory infections in the first year of life. Upon initial examination at our institute at 12 months, he was noted to be very irritable and stiff with marked spastic quadriplegia. He was delayed in all milestones and had strabismus. Head circumference and weight were below the 2nd centile, whereas height was at the 10th centile. Over the years he continued to have poor growth, feeding difficulties necessitating a gastrostomy tube placement at age four years, recurrent aspirations and pneumonias necessitating tracheotomy placement at age four years, constipation, severe neuromuscular scoliosis necessitating spinal fusion, seizures, microcephaly, spastic quadriplegia, and profound intellectual disability. Upon most recent examination at 21 years, he was non-verbal and unable to follow commands, walk or sit independently. He could visually track and grab after objects with his hands. He had a social smile. He had strabismus and nystagmus. He had choreiform movements of his upper extremities. He had muscle atrophy, spasticity, and contractures of all four extremities. His head circumference was 51.5 cm (<2nd centile), and height and weight were <1st centile. He had dysmorphic craniofacial features including prognathia, midface retrusion, short philtrum, and large and prominent ears. Brain MRI at 19 years showed extensive atrophy of the bilateral frontal lobes extending all the way down to the perisylvian fissure, mild frontal ventriculomegaly, and thinning of the corpus callosum. SNP chromosomal microarray was normal. He continues to have multiple generalized seizures per week that have been refractory to multiple antiepileptic medications. Seizures seem to be precipitated by constipation. A trial of topiramate at age 21 led to increased alertness, but this was discontinued due

to severe insomnia. EEGs have shown diffuse cerebral disturbance without electrographic correlates to his seizures.

Proband 3

The third proband is a four-year-old boy, born to healthy, non-consanguineous parents. He first came to medical attention at birth when he was born at 34 weeks gestation due to maternal pre-eclampsia and gestational diabetes with normal birth measurements. Shortly after birth he was noted to have hypertonia, nystagmus and an increased startle reflex. He was diagnosed with optic nerve hypoplasia, and exhibited seizure-like episodes at the age of 14 months and developmental delay. He was examined the last time in May 2015 at almost five years of age. He was globally delayed, and at our last visit he was able to take steps using a walker or holding onto one hand. He had no words and had little receptive language; no regression was reported. His growth parameters were normal with a height of 113.5 cm, weight of 20 kg and head circumference of 52.2 cm. His brain MRI in 2011 was normal aside from the optic nerves. He was being treated for spasticity in his upper and lower extremities. His EEGs have demonstrated generalized slowing without epileptiform discharges. Chromosome oligoarray, testing for Fragile X syndrome and testing for the *GLRB, GLRA1*, and *SLC6A5* genes was normal.

Proband 4

The fourth proband is a four-year-old boy, born to healthy, non-related Caucasian parents. He was born after an uneventful pregnancy with normal birth weight, height and head circumference. After an uneventful early development, he first came to attention with a febrile seizure at the age of one year and one month. An EEG at this time showed generalized spikes and waves during sleep, indicating an increased susceptibility to seizures. Despite of valproate therapy and an improvement in the EEG, the boy showed further prolonged febrile seizures and two episodes of status epilepticus. Milestones of motor development were delayed: sitting at 15 months and walking without support at 20 months. At the age of three years, his gait was clumsy and unstable. He had mild muscular

hypotonia. The speech development was severely impaired. He spoke two words since the age of two years without a substantial improvement of expressive language. His receptive language was also severely impaired. Intellectually disability was evident and he showed abnormal behaviour including hyperactivity, severely reduced attention span, stereotypic hand movements, aggressive behaviour, and reduced interaction. In addition, he suffered from recurrent ear infections in the first three years of life. At last examination at age of four years, he was a severely disabled boy without expressive language, with impaired receptive language and a clumsy gait. His behaviour problems had improved. He started to communicate non-verbally and was able to focus and to play. Seizures occurred rarely under treatment and were always with a background of fever. The measurements were in the normal range and EEG showed no epileptic discharges.

Extensive metabolic work up, brain MRI, ultrasound of abdomen, and eye examination yielded normal results. Conventional chromosome analysis of lymphocytes, array-CGH, *SNRPN*-locus methylation analysis, direct sequencing of *SCN1A*, Fragile X analysis, and epileptic encephalopathy panel analysis of 78 genes gave normal results.

Proband 5

The fifth proband is a four-year-old girl, born to healthy, non-consanguineous parents. The pregnancy and birth were unremarkable. The measurements of the girl were normal at birth and postnatal growth was unremarkable. Around the third birthday, a delayed speech development was clinically diagnosed. At the time of examination, the girl was four years old, showed normal growth parameters, and could bilingually speak single words and few simple two-word sentences. However, most of the expressive language was incomprehensible, while she understood the majority of the information given by her mother or other people, thus presenting a discrepancy between receptive and expressive lingual abilities. Her motor development was in the normal range. Apart from hyporeflexia and a simian crease on both hands, she did not show any physical abnormalities or malformations. She showed mild social behaviour abnormalities; she was friendly, showed a lack of distance towards adults but reduced interaction with other children at the same age. In addition, she

presented a reduced attention span. She visits an integrative kindergarten and receives occupational therapy and physiotherapy. Tests for autism and hearing loss as well as EEG and brain MRI were unremarkable. Karyotyping and array analysis excluded chromosomal abnormalities.

Supplemental Note: Exome Sequencing

In all five cases, trio exome sequencing was performed.

DNA from the proband 1 and his parents were subjected to exome capture using NimbleGen SeqCap EZ MedExome (Roche, Wisconsin, USA), followed by sequencing on an Illumina NextSeq550 (Illumina, San Diego, CA, USA). Raw reads were aligned using the Burrows-Wheeler Alignment tool (BWA-MEM) v. 0.7.15[1] and the GATK Best Practice pipeline v. 3.8–0 was used for variant calling[2]. Annotation and filtering of variants was performed using VarSeq 1.4.6 (Golden Helix).

Exome sequencing for the probands 2 and 3 was performed as a trio including both biological parents by Ambry Genetics Laboratory. Samples were prepared using the NimbleGen SeqCap EZ VCR (Roche, Wisconsin, USA) and sequenced on the Illumina HiSeq2000 Sequencer (Illumina, San Diego, CA, USA). Data annotation and interpretation were performed as previously reported[3].

For proband 4 and his parents, exome sequencing was performed in a trio mode as described before[4]. Enrichement of coding DNA fragments was done with a SureSelect Human All Exon 50Mb V5 Kit (Agilent, Santa Clara, CA, USA) and sequencing was performed on a HiSeq2500 system (Illumina, San Diego, CA, USA). Alignment of the reads was done to the GRCh37 (hg19) human reference genome with the help of the Burrows-Wheeler Aligner (BWA, v.0.5.87.5). SAMtools (v.0.1.18), PINDEL (v. 0.2.4t), and ExomeDepth (v.1.0.0) were used to detect genetic variation.

For proband 5, exome capture and sequencing was performed in a trio-mode as described before[5] with use of Illumina´s Nextera Rapid Capture Exome Kit (Illumina, Inc., San Diego, CA, USA) and the NextSeq500 sequencer (Illumina, San Diego, CA, USA) in Centogene´s laboratory (Rostock, Germany). The raw-sequencing reads were converted and inserted in an in-house pipeline of Centogene for the analysis of data out of whole-exome-sequencing. By utilising the mem algorithm in the bwa software, short-reads were aligned to the GRCh37 (hg19) human reference genome. Three different tools, GATK HaplotypeCaller, freebayes, and samtools, were used. Filtering the variants was performed based on annotated excel tables as well as using VarvisTM from LimbusTM (https://limbus-medtec.com/).

Supplemental Tables

Table S1: Variants identified in *GRIA4* (NM_000829.3)

	Proband 1	Proband 2	Proband 3	Proband 4	Proband 5
HGVS DNA reference	c.1915A>T	c.1921A>G	c.1928C>G	c.1931C>T	c.2090G>C
HGVS protein reference	p.(Thr639Ser)	p.(Asn641Asp)	p.(Ala643Gly)	p.(Ala644Val)	p.(Arg697Pro)
Genomic position	11:105,797,534A>T	11:105,797,540A>G	11:105,797,547C>G	11:105,797,550C>T	11:105,804,491G>C
inheritance	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*
SIFT	Deleterious (score: 0)	Deleterious (score: 0)	Deleterious (score: 0)	Deleterious (score: 0)	Deleterious (score: 0.04)
MutationTaster	Disease causing (p=1)	Disease causing (p=1)	Disease causing (p=1)	Disease causing (p=1)	Disease causing (p=0.986)
Polyphen-2	Probably damaging (score=1.0)	Probably damaging (score=1.0)	Probably damaging (score=1.0)	Probably damaging (score=1.0)	Benign (score=0.026)
phyloP (ucsc)	phyloP: 5.13	phyloP: 5.13	phyloP: 6.26	phyloP: 6.26	phyloP: 2.71
ScoreGERP++	5.76	5.76	5.76	5.76	4.25
CADDphred (wannovar)	26.3	26.3	34	36	14.51
REVEL	0.669	0.669	0.663	0.751	0.1924

Table S2: Example on identified variants that are rare (<0.01% for de novo and <1% for the rest) in families 1 and 5

Family 1	Chromosome	Genomic Position	Reference	Variant	Gene	Transcript	cDNA Change	Protein Change
de novo	chr11	105797534	A	T	*GRIA4*	NM_000829.3	c.1915A>T	p.Thr639Ser
Hemizygous	chrX	83419374	C	G	*RPS6KA6*	NM_014496.4	c.103G>C	p.Asp35His
compound heterozygous	chr19	36211409	C	T	*KMT2B*	NM_014727.2	c.1160C>T	p.Ala387Val
compound heterozygous	chr19	36216114	-	C	*KMT2B*	NM_014727.2	c.3529-8_3529-7insC	?
compound heterozygous	chr17	39724752	C	A	*KRT9*	NM_000226.3	c.1170+8G>T	?
compound heterozygous	chr17	39728000	C	T	*KRT9*	NM_000226.3	c.245G>A	p.Ser82Asn
compound heterozygous	chr8	145737131	C	G	*RECQL4*	NM_004260.3	c.3435G>C	p.Gln1145His
compound heterozygous	chr8	145740707	C	T	*RECQL4*	NM_004260.3	c.1390+3G>A	?

Family 5	Chromosome	Genomic Position	Reference	Variant	Gene	Transcript	cDNA Change	Protein Change
de novo	chr11	105804491	G	C	*GRIA4*	NM_000829.3	c.2090G>C	p.Arg697Pro
Homozygous	chr3	193051624	C	T	*ATP13A5*	NM_198505.2	c.1187G>A	p.Ser396Asn
compound heterozygous	chr1	159898074	C	T	*IGSF9*	NM_001135050.1	c.3104G>A	p.Arg1035Gln
compound heterozygous	chr1	159912852	G	T	*IGSF9*	NM_001135050.1	c.148C>A	p.Pro50Thr
compound heterozygous	chr1	183079729	C	T	*LAMC1*	NM_002293.3	c.961C>T	p.Pro321Ser
compound heterozygous	chr1	183091040	G	A	*LAMC1*	NM_002293.3	c.2173G>A	p.Ala725Thr
compound heterozygous	chr13	23907033	G	A	*SACS*	NM_014363.5	c.10982C>T	p.Ala3661Val
compound heterozygous	chr13	23907106	T	C	*SACS*	NM_014363.5	c.10909A>G	p.Met3637Val
compound heterozygous	chr7	1510791	G	A	*INTS1*	NM_001080453.2	c.6325C>T	p.Arg2109Cys
compound heterozygous	chr7	1521087	G	C	*INTS1*	NM_001080453.2	c.3741C>G	p.Phe1247Leu
compound heterozygous	chr7	149519733	G	A	*SSPO*	NM_198455.2	c.13237G>A	p.Ala4413Thr
compound heterozygous	chr7	149523544	C	T	*SSPO*	NM_198455.2	c.14474C>T	p.Pro4825Leu

Filtering the variants in families 1 and 5 revealed only one *de novo* variant (in *GRIA4*) each that potentially impacts the protein structure. As we filtered for autosomal recessive inheritance modus (homozygous and heterozygous) and rare variants (<1%), we identified few variants in each family. These, however, did not fulfil further criteria and could be excluded. Also in family 4, there were no further *de novo* variants and additionally no other relevant candidate variants for an autosomal recessive inheritance modus. For the other two families, number 2 and 3, different evaluation pipelines in a diagnostic setting were used. Also in these two families we could get certain that no other relevant *de novo* variants and no other candidate variants were found.

Table S3: Genes encoding the glutamate receptor subunits and variants in their SYTANLAAF motif

Gene	Transcript NM	Amino acid position of SYTANLAAF	Described pathogenic variants in SYTANLAAF	References
GRIA1	NM_001114183.1	629-637	p.(Ala636Thr)	de Ligt et al., 2012[6]; Geisheker et al., 2017[7]
GRIA2	NM_001083619.1	636-644	None	
GRIA3	NM_000828.4	647-655	p.(Ala653Thr)	Davies et al., 2017[8]
GRIA4	NM_000829	637-645	p.(Thr639Ser), p.(Asn641Asp), p.(Ala643Gly), p. (Ala644Val)	This study
GRIN1	NM_007327	646-654	p.(Tyr647Cys), p.(Tyr647Ser), p.(Asn650Lys), p.(Ala653Gly)	Allen et al., 2013[9]; Lemke et al., 2016[10]; Ohba et al., 2015[11]
GRIN2A	NM_000833.4	644-652	p.(Asn648Ser), p.(Leu649Pro), p.(Leu649Val), p.(Phe652Val)	de Ligt et al., 2012[6]; Lesca et al., 2013[12]
GRIN2B	NM_000834.3	642-650	None	
GRIN2C	NM_000835	642-650	None	
GRIN2D	NM_000836	672-680	None	
GRIN3A	NM_133445	759-767 (TYTANLAAV)	None	
GRIN3B	NM_138690	659-667 (SYTANLAAV)	None	
GRIK1	NM_000830.3	665-673	None	
GRIK2	NM_021956.4	650-658	None	
GRIK3	NM_000831.3	652-660	None	
GRIK4	NM_014619.4	635-643	None	
GRIK5	NM_002088	634-642	None	
GRID1	NM_017551.2	647-655	None	
GRID2	NM_001510.3	647-655	p.(Ala654Asp), p.(Ala654Thr)	Coutelier et al., 2015[13]

References: 6, 7, 8, 9, 10, 11, 12, 13

Table S4: Structural destabilization of GRIA4 by interfacial variants

Variant	Energy(kcal)
T639S	0.36
N641C	0.84
N641D	0.80
N641K	-0.55
A643G	0.77
A644V	0.18
R697P	7.17
R697Q	0.16

Supplemental References

1. Li, H. (2013). Aligning sequence reads, clone sequences and assembly contains with BWA-MEM. Oxford University Press; 2014. pp. 1–3. http://arxiv.org/abs/1303.3997. 2013
2. Van der Auwera, G.A., Carneiro, M.O., Hartl, C., Poplin, R., Del Angel, G., Levy-Moonshine, A., Jordan, T., Shakir, K., Roazen, D., Thibault, J., et al. (2013). From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Curr Protoc Bioinformatics. *43*, 11.10.1–11.10.33
3. Farwell, K.D., Shahmirzadi, L., El-Khechen, D., Powis, Z., Chao, E.C., Tippin Davis, B., Baxter, R.M., Zeng, W., Mroske, C. and Parra, M.C., et al. (2015). Enhanced utility of family-centered diagnostic exome sequencing with inheritance model-based analysis: results from 500 unselected families with undiagnosed genetic conditions. Genet Med. *17,* 578–586.
4. Hempel, M., Cremer, K., Ockeloen, C.W., Lichtenbelt, K.D., Herkert, J.C., Denecke, J., Haack, T.B., Zink, A.M., Becker, J. and Wohlleber, E., et al. (2015). De Novo Mutations in CHAMP1 Cause Intellectual Disability with Severe Speech Impairment. Am J Hum Genet. *97,* 493–500.
5. Trujillano, D., Bertoli-Avella, A.M., Kumar Kandaswamy, K., Weiss, M.E., Koster, J., Marais, A., Paknia, O., Schroder, R., Garcia-Aznar, J.M. and Werber, M., et al. (2017). Clinical exome sequencing: results from 2819 samples reflecting 1000 families. Eur J Hum Genet. *25,* 176–182.
6. Ligt, J. de, Willemsen, M.H., van Bon, Bregje W M, Kleefstra, T., Yntema, H.G., Kroes, T., Vulto-van Silfhout, A.T., Koolen, D.A., Vries, P. de and Gilissen, C., et al. (2012). Diagnostic exome sequencing in persons with severe intellectual disability. N Engl J Med. *367,* 1921–1929.
7. Geisheker, M.R., Heymann, G., Wang, T., Coe, B.P., Turner, T.N., Stessman, H.A.F., Hoekzema, K., Kvarnung, M., Shaw, M. and Friend, K., et al. (2017). Hotspots of missense mutation identify neurodevelopmental disorder genes and functional domains. Nat Neurosci. *20,* 1043-1051
8. Davies, B., Brown, L.A., Cais, O., Watson, J., Clayton A.J., Chang, V.T., Biggs, D., Preece, C., Hernandez-Pliego, P., Krohn, J. (2017). A point mutation in the ion conduction pore of AMPA

receptor GRIA3 causes dramatically perturbed sleep patterns as well as intellectual disability. Human Molecular Genetics, ddx270

9. Epi4K Consortium, Epilepsy Phenome/Genome Project, Allen, A.S., Berkovic, S.F., Cossette, P., Delanty, N., Dlugos, D., Eichler, E.E., Epstein, M.P., Glauser, T., Goldstein, D.B. and Han, Y., et al. (2013). De novo mutations in epileptic encephalopathies. Nature *501*, 217–221.
10. Lemke, J.R., Geider, K., Helbig, K.L., Heyne, H.O., Schütz, H., Hentschel, J., Courage, C., Depienne, C., Nava, C. and Heron, D., et al. (2016). Delineating the GRIN1 phenotypic spectrum: A distinct genetic NMDA receptor encephalopathy. Neurology *86,* 2171–2178.
11. Ohba, C., Shiina, M., Tohyama, J., Haginoya, K., Lerman-Sagie, T., Okamoto, N., Blumkin, L., Lev, D., Mukaida, S. and Nozaki, F., et al. (2015). GRIN1 mutations cause encephalopathy with infantile-onset epilepsy, and hyperkinetic and stereotyped movement disorders. Epilepsia *56,* 841–848.
12. Lesca, G., Rudolf, G., Bruneau, N., Lozovaya, N., Labalme, A., Boutry-Kryza, N., Salmi, M., Tsintsadze, T., Addis, L. and Motte, J., et al. (2013). GRIN2A mutations in acquired epileptic aphasia and related childhood focal epilepsies and encephalopathies with speech and language dysfunction. Nat Genet. *45*, 1061–1066.
13. Coutelier, M., Burglen, L., Mundwiller, E., Abada-Bendib, M., Rodriguez, D., Chantot-Bastaraud, S., Rougeot, C., Cournelle, M.-A., Milh, M. and Toutain, A., et al. (2015). GRID2 mutations span from congenital to mild adult-onset cerebellar ataxia. Neurology *84,* 1751–1759.