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

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Using trio whole-exome sequencing, we have identified de novo heterozygous pathogenic variants in GRIA4 in five unrelated individuals with intellectual disability and other symptoms. GRIA4 encodes an AMPA receptor subunit known as GluR4, which is found on excitatory glutamatergic synapses and is important for learning and memory. Four of the variants are located in the highly conserved SYTANLAAF motif in the transmembrane protein M3, and the fifth is in an extra-cellular domain. Molecular modeling of the altered protein showed that three of the variants in the SYTANLAAF motif orient toward the center of the pore region and most likely lead to disturbance of the gating mechanism. The fourth variant in the SYTANLAAF motif most likely results in reduced permeability. The variant in the extracellular domain potentially interferes with the binding between the monomers. On the basis of clinical information and genetic results, and the fact that other subunits of the AMPA receptor have already been associated with neurodevelopmental disorders, we suggest that pathogenic de novo variants in GRIA4 lead to intellectual disability with or without seizures, gait abnormalities, problems of social behavior, and other variable features.

Intellectual disability (ID) has a prevalence of about 1%[1] and is characterized by substantial limitations in both intellectual functioning and adaptive behavior starting before the age of 18 years (ICD-10 2016, World Health Organization). Recent studies have shown that de novo variants are a frequent cause of neurodevelopmental disorders.[2]

At five centers in Germany, Denmark, and the United States, we clinically examined five unrelated individuals with neurodevelopmental disorders, primarily ID. In addition, four of the five affected individuals had seizures or abnormal electroencephalography (EEG). Further symptoms were muscular hypertonia followed by spasticity in later age, abnormal brain MRI, and different behavioral disorders (Table 1 and Supplemental Note: Case Reports).

This study was approved by the ethics committees of the University of Leipzig (402/16-ek) and the University Medical Center of Hamburg-Eppendorf (PV3802). Informed consent was obtained from all examined individuals or their guardians. Otherwise, testing was done as part of routine clinical care, and therefore institutional ethics approval was not required. All families provided informed consent for clinical testing and publication.

We performed exome sequencing for all five individuals as a trio analysis including DNA samples of both biological parents. DNA from the proband and the parents was subjected to exome capture by NimbleGen SeqCap EZ MedExome (Roche), NimbleGen SeqCap EZ VCR (Roche), SureSelect Human All Exon 50Mb V5 (Agilent), or the Nextera Rapid Capture Exome Kit (Illumina). Sequencing was performed on an Illumina NextSeq 500, NextSeq 550, HiSeq 2000, or HiSeq 2500. For proband 1, raw reads were aligned with the Burrows-Wheeler Aligner (BWA-MEM) v.0.7.15,[3] and the Genome Analysis Toolkit Best Practices pipeline v.3.8-0 was used for variant calling.[4] Annotation and filtering of variants was performed with VarSeq 1.4.6 (Golden Helix) for proband 1 and his parents. For the other four probands, bioinformatic preparation of the data and data annotation and interpretation were performed with house-made pipelines as previously reported (see Supplemental Note: Exome Sequencing).[5–7] Variants of interest were confirmed by dideoxy sequencing, and cosegregation analysis was performed in all informative, available family members. This revealed de novo missense variants in GRIA4 (MIM: 138246) in the five probands: c.1915A>T (p.Thr639Ser), c.1921A>G (p.Asn641Asp), c.1928C>G (p.Ala643Gly), c.1931C>T (p.Ala644Val), and c.2090G>C (p.Arg697Pro) (see Table S1). Further protein-changing, de novo variants were not identified in any of the families (see also Table S2). None of the variants in GRIA4 have been observed in gnomAD[8] (accessed October 2017) or the 1000 Genomes Project.[9] Most of the in silico programs predicted the variants to be

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Table 1. Clinical Features of Individuals with Predicted Deleterious Variants in *GRIA4*

	Proband				
	1	**2**	**3**	**4**	**5**
Age at last examination	15 years	21 years	4 years	4 years	4 years
Sex	male	male	male	male	female
HGVS DNA reference	c.1915A>T	c.1921A>G	c.1928C>G	c.1931C>T	c.2090G>C
Variant	p.Thr639Ser	p.Asn641Asp	p.Ala643Gly	p.Ala644Val	p.Arg697Pro
Prenatal period	unremarkable pregnancy after IVF	unremarkable	maternal pre-eclampsia	unremarkable	unremarkable
Gestational week at birth	40	40	34	39	40
Birth parameters	weight 3,755 g (62P), length 53 cm (59P), OFC not known	weight 3,320 g (25P), length and OFC not known	weight 3,033 g (95P), length 47 cm (61P), OFC 34 cm (86P)	weight 3,450 g (47P), length 53 cm (68P), OFC 37 cm (90P)	weight 4,150 g (95P), length 53 cm (72P), OFC 36 cm (80P)
Neonatal period	stiffness (stiff baby syndrome), irritability, excessive startle reflex	irritability, stiffness	hypertonia, nystagmus, increased startle reflex	unremarkable	unremarkable
Congenital anomalies	none	none	none	none	none
Postnatal growth	unremarkable	failure to thrive, short stature, microcephaly	unremarkable	unremarkable	unremarkable
Developmental delay	mild to moderate	severe	severe	moderate to severe	mild to moderate
Speech	speech with dysarthria	non-verbal communication	non-verbal communication	non-verbal communication	single words and a few two-word sentences
Brain MRI	unremarkable	bilateral symmetric extensive atrophy of frontal lobes, mild frontal ventriculomegaly, thin corpus callosum	optic nerve hypoplasia	unremarkable	unremarkable
Social behavior	interaction with adults, reduced attention span, tension with irritability and anxiety	social smile, interaction with caregivers	occasional response to voice	3 years: hyperactivity, reduced attention span, aggressive behavior, reduced interaction with other children; 4 years: non-verbal communication, ability to focus and play	lack of distance toward adults, reduced interaction with other children, strong searching for physical contact, mood changes with aggressive behavior and attention deficits
Muscle tone	hyperekplexia with exaggerated head-retraction reflex, stiffness, and hypertonia	severe spastic quadriplegia and hypertonia with contractures	spasticity	mild muscular hypotonia (neonatal)	unremarkable
Walking abilities	difficulties when walking in a straight line, stiff gait, ability to run	inability to walk	supported walking	clumsy gait	yes
Seizures	no, but severe contraction burst in relation to trauma	intractable generalized seizures, onset at 5 weeks	seizure-like episodes, onset at 14 months	febrile seizures at 13 months	no

Table 1. Continued

	Proband				
	1	2	3	4	5
Craniofacial dysmorphism	large ears	prognathism, midface retrusion, short philtrum, large ears	large ears	no	no
EEG	unremarkable	diffuse cerebral disturbance without electrographic correlates to the seizures	generalized slowing, no epileptiform discharges	generalized spikes and waves during sleep	unremarkable
Additional features or notes	sleeping problems in childhood	bilateral hiatal hernias, gastresophageal reflux, feeding difficulties, apneas, recurrent respiratory infections in first year of life, strabismus, choreiform movements	–	stereotypic hand movements	hyporeflexia, simian crease on both hands

Abbreviations are as follows: IVF, in vitro fertilization; OFC, occipitofrontal circumference; MRI, magnetic resonance imaging; and P (e.g., 62P), percentile.

pathogenic, however inconstantly. In addition, GRIA4 is intolerant of loss-of-function variants (pLI = 0.99) and missense variants (Z score = 3.16).[8]

GRIA4 encodes GluR4, a subunit of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor.[10,11] These glutamate-dependent receptors consist of the four subunits GluR1, GluR2, GluR3, and GluR4[12] and have an important function in synaptic transmission, activity-dependent synaptic plasticity, and the control of network activity.[13] AMPA receptors belong to the group of glutamate-gated cationic ion channels and are important for various neurologic functions, such as synaptic communication[11] and long-term potentiation.[14] AMPA receptors (encoded by GRIA1 [MIM: 138248], GRIA2 [MIM: 138247], GRIA3 [MIM: 305915], and GRIA4) share vast similarities with other ionotropic glutamate receptors, comprising kainate receptors (encoded by GRIK1 [MIM: 138245], GRIK2 [MIM: 138244], GRIK3 [MIM: 138243], GRIK4 [MIM: 600282], and GRIK5 [MIM: 600283]), NMDA receptors (encoded by GRIN1 [MIM: 138249], GRIN2A [MIM: 138253], GRIN2B [MIM: 138252], GRIN2C [MIM: 138254], GRIN2D [MIM: 602717], GRIN3A [MIM: 606650], and GRIN3B [MIM: 606651]), and δ receptors (encoded by GRID1 [MIM: 610659] and GRID2 [MIM: 602368]).[12,15] Pathogenic variants in several of these genes have been associated with developmental delay, ID, different epilepsy disorders (including epileptic encephalopathy), and ataxia.[16–35]

Subunits GluR1–GluR4 of the AMPA receptor can form both homo- and heteromers but are usually heteromeric.[12,36] The relative combination ratios of the four subunits varies in different types of neurons,[36] and the conductance properties of the receptors are dependent on the assembly of their subunits.[37] GRIA1 encodes GluR1 and is seen as an important element in associative memory formation.[38] GRIA1 has been associated with ID and neurodevelopmental disorders due to a recurrent de novo missense variant, c.1906G>A (p.Ala636Thr).[24,28] GRIA2 encodes GluR2. It has been shown that receptors lacking GluR2 have a higher Ca^{2+} permeability and channel conductance (reviewed by Isaac et al.[39]). A deletion comprising exons 1 and 2 of GRIA2 has been described as a possible cause of ID (and gait abnormalities and abnormal behavior),[40] and an in-frame deletion in GRIA2 has been described in a person with ID.[2] Pathogenic variants in GRIA3 have been associated with X-linked mental retardation 94 (MIM: 300699). Different variants are described in the literature in persons with ID, seizures, autistic features, short stature, and behavioral problems.[16,27,29,30] GRIA4, the main subject of this study, has not yet been reported in association with any disorder. In rat brains, the subunit GluR4, encoded by Gria4, is found ubiquitously in the CNS, but its amount is relatively high in CA1 pyramidal cells and the dentate gyrus of the hippocampus, layers III and IV of the cerebral cortex, and the granule cells of the cerebellum.[41] In the hippocampus, GluR4 was more abundant only in the first postnatal

Figure 1. Structure of GRIA4 and Variant Location
In addition, we show the allele count of genetic variants reported by gnomAD[8] for the canonical transcript ENST00000282499 (GenBank: NM_000829.3). At position Arg697, where we describe the variant p.Arg697Pro in proband 5 in this report, another variant (p.Arg697Gln) is reported in gnomAD.[8] In the main text, we describe the most likely mild impact of this change on the function of the protein. The figure was made with the help of Protter.[64]

week.[42] Sagata et al.[11] produced *Gria4*−/− mice, which showed 10% lower weight than wild-type mice, normal anxiety-like behavior in a novel environment, muscle weakness, and more faults in the training period for the hippocampus-dependent Barnes circular maze test, which proofs spatial reference memory. In addition, these knockout mice showed normal spontaneous locomotor activity, and the authors suggest that their social behavior was improved. The authors concluded that the *Gria4*−/− mice were impaired solely in the acquisition of spatial reference memory and not the retention of spatial reference memory.[11] However, Beyer et al.[36] demonstrated that mice with a genetic deficiency of GRIA4, either resulting in reduced function or its complete absence, showed highly frequent spike-wave discharges on EEG, which are associated with absence epilepsy. In addition, some of the mice homozygous for the knockout allele developed a mild cerebellar ataxia.[36] Notably, in both studies,[11,36] the modification of GRIA4 was toward loss of function. However, the identified variants in the present report are heterozygous and do not cause loss of function per se.

The variants p.Thr639Ser, p.Asn641Asp, p.Ala643Gly, and p.Ala644Val in probands 1, 2, 3, and 4, respectively, are located in the SYTANLAAF motif. AMPA and other glutamate receptors have three transmembrane domains (M1, M3, and M4) plus the re-entrant membrane loop M2[43] (Figure 1). The M2 loop and the M3 segment represent the major pore-lining domains in GluRs.[44] The channel transition between symmetries possibly occurs at the M3 segment.[45] This region contains the highly conserved

SYTANLAAF motif,[46] which has the strictest amino acid conservation among all members of the ionotropic glutamate receptor family.[47] It is known that the region moves in response to activation and that a distinction among channel types is the degree of exposure.[48,49] Changes in the SYTANLAAF motif at Thr639 and Ala643[46,50] are associated with constitutively open channels.[51] Several other studies have also proved that other changes in the SYTANLAAF motif lead to a permanently open channel (e.g., Chang and Kuo[46]) in the sense of gain of function.[52–54] Human *de novo* missense variants affecting this motif have already been described as disease causing in several genes encoding ionotrophic glutamate receptors: *GRIN1*, *GRIN2A*, and *GRID2*.[18,24,26,55] Also, a recurrent *de novo* variant in *GRIA1* affects this motif in a person with ID[24,28] (see Table S3). In addition, Li et al. reported a functionally proven gain-of-function *de novo* variant in *GRIN2D*, c.1999G>A (p.Val667Ile), affecting the encoded protein very close to the SYTANLAAF motif in two unrelated persons with epileptic encephalopathy.[22]

The alanine residues at positions 643 and 644 orient toward the center of the pore region and most likely inhibit closing of the channel either by trapping the channel in the open state or allowing leakage, thus resulting in constitutive opening of the channel.[56] Like p.Ala643Gly, the change from threonine to serine at position 639 removes a methyl group that partially occludes the closed channel, most likely resulting in a partially open or leaky channel in the closed state. As Table S4 shows, variants at Ala643 and Ala644 introduce moderate steric perturbations. However, they
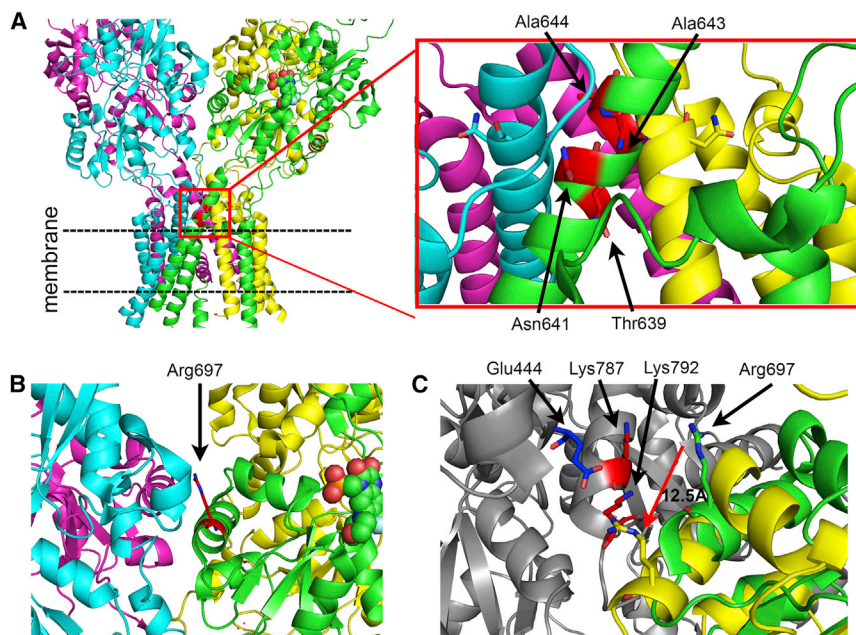
Figure 2. Tetrameric Structure of GRIA4 with Variants and Allosteric Movements

The structure of GRIA4 was built off the homologous structure of GRIA2 for *Rattus norvegicus* in the complex with an agonist (PDB: 3KG2[45]). The homology model of Gria4 was built off the homology modeling module of ROSETTA,[60] where the sequence alignments were generated with TCOFFEE.[61,62] The energy calculations of both and the destabilization of internal structure were calculated with the FoldX program.[63] The images were generated with PyMOL (PyMOL Molecular Graphics System, v.1.8, Schrödinger).

(A) GRIA4 tetramer with residues in the SYTANLAAF motif of M3. The residue positions of missense variants are indicated by red sticks.

(B) GRIA4 tetramer with a variant in the ligand binding domain. The residue position of wild-type Arg697 is indicated by red sticks.

(C) Arg697 moves by more than 10 Å from the ligand-bound (green; PDB: 3KG2[10]) to the *apo* (yellow; PDB: 4U2P[62]) state. Arg697 interacts with residues on the adjacent monomer (gray), including the repulsive Lys787 and Lys792 (red sticks) in the ligand-bound state and the attractive Glu444 (blue sticks) in the *apo* state.

significantly affect the function of the channel by disrupting the interactions between monomers in the membrane and disrupting the gating and transport of ions through the channel. In addition, the variant c.1931C>T (p.Ala644Val) in proband 4 is at the same position in the motif as the pathogenic variants in GRIA1 (p.Ala636Thr)[24,28] and GRID2 (p.Ala654Asp and p.Ala654Thr),[26] as well as in the functionally well-studied Lurcher mouse model. The Lurcher variant (SYTANLAAF to SYTANLATF in the GluRδ2 receptor) in one subunit of a heteromeric glutamate receptor is functionally dominant, leads to spontaneous channel activity, and slows channel kinetics, even though the changes in gating kinetics are increased in homomeric Lc-mutated forms.[52]

Conversely, for the known variants p.Asn641Lys and p.Asn641Cys, variants at the equivalent position in the paralog GRIA2 (p.Asn619Lys and p.Asn619Cys) are associated with significant loss of permeability.[57] Our molecular modeling showed that residue Asn641 orients toward the adjacent monomers, interacts with the backbone atoms of the nearby loops, and thus stabilizes the open and closed states and most likely mediates the transition between states. Loss of this functionality either inhibits the transition or allows the channel to become trapped in an inactivated state.

The fifth variant in this study is c.2090G>C, leading to the protein change p.Arg697Pro, which is located outside the SYTANLAAF motif. Pathogenic variants leading to protein changes outside of the SYTANLAAF sequence have already been described in association with ID, different epilepsy disorders, or ataxia in the glutamate receptor genes *GRIA3*, *GRIN1*, *GRIN2A*, *GRIN2B*, *GRIN2D*, *GRID2*, and *GRIK2*.[16–27,29–34,55] In the homologous segments of the protein sequence around our variant

p.Arg697Pro, pathogenic variants (e.g., the two variants in GluR3, p.Met706Thr [c.2117T>C] and p.Gly721Arg [c.2161G>A]) have also been identified.[16,27] The region around Arg697 lies at the interface between monomers in the tetramer within the ligand binding domain (Figure 2), which is involved in agonist and antagonist binding and activation of the transmembrane domain.[58] The residue itself is partially exposed and does not appear to engage in strong interactions with residues on adjacent monomers. It should be noted that between the homology models of the bound (PDB: 3KG2[45]) and apo (PDB: 4U2P[59]) states, Arg697 moves by more than 10 Å (see Figure 2). Additionally, the conformation for the ligand-bound state places Arg697 near repulsive side chains on Lys787 and Lys792, whereas in the apo state it interacts favorably with Glu444 of the adjacent monomers, thereby stabilizing the apo state. The variant identified in this study, p.Arg697Pro, leads to the insertion of a helix breaker in the middle of an α helix at the interface between monomers. So, p.Arg697Pro leads to the disruption of the α helix and potentially to local unwinding, which in turn interferes with binding between monomers. Additionally, the loss of the charged sidechain disturbs the equilibrium between the two states. This is not the case for variant p.Arg697Gln, which has an allele frequency of 0.00002 in gnomAD[8] (see Figure 1). The destabilization energy of p.Arg697Gln is small (0.17 kcal), most likely indicating that the change does not strongly disrupt protein function. Variant p.Arg697Gln has a more subtle effect and could interfere at an intermediate stage in monomer folding or tetramerization and most likely affects functionality more mildly such that it might be imperceptible from baseline variations.

The importance of GRIA4 in neuronal function, the positions of the identified variants and their predicted effects on the protein, and the previously published data on the other subunits of the AMPA receptor and other glutamate receptors let us consider the *de novo* variants in *GRIA4* in this study as causative for the phenotypes of our probands. The data that we have let us assume that the pathogenic effect is due to a dominant functional effect and not loss of function. This is in line with other pathogenic variants in glutamate receptors. In addition, the positions of our variants and the executed molecular modeling suggest some degree of genotype-phenotype correlation: a mild phenotype of the girl with the p.Arg697Pro variant outside the SYTANLAAF-motif and a more severe phenotype for variants within the motif. Also, within the motif, the variant at position 639 (which has a lesser effect on the structural destabilization of GRIA4) is in an individual with milder symptoms. However, this still requires further analysis and the identification of additional affected individuals. In conclusion, we suggest *de novo*, heterozygous pathogenic variants in *GRIA4* as causative for ID with or without seizures and gait abnormalities.

Accession Numbers

The accession numbers for the *GRIA4* variants reported in this paper are ClinVar: SCV000611121, SCV000611122, SCV000611123, SCV000611124, SCV000611125, and SCV000611126.

Supplemental Data

Supplemental Data include two Supplemental Notes and four tables and can be found with this article online at https://doi.org/10.1016/j.ajhg.2017.11.004.

Conflicts of Interest

A.C., D.N.S., and K.L.H. are employed by and receive a salary from Ambry Genetics. A.F. is a consultant to Ambry Genetics. J.S.C. is a consultant to Invitae. Exome sequencing is a commercially available test.

Web Resources

ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/
GenBank, https://www.ncbi.nlm.nih.gov/genbank/
Genome Aggregation Database (gnomAD), http://gnomad.broadinstitute.org/
HGMD, http://www.hgmd.cf.ac.uk/ac/index.php
MutationTaster, http://www.mutationtaster.org/
OMIM, http://omim.org/
PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/
Protter, http://wlab.ethz.ch/protter/
PubMed, https://www.ncbi.nlm.nih.gov/pubmed/
REVEL: Rare Exome Variant Ensemble Learner, https://sites.google.com/site/revelgenomics/
RSCB Protein Data Bank, https://www.rcsb.org/pdb/home/home.do
UCSC Genome Browser, https://genome.ucsc.edu/
Varvis, https://www.limbus-medtec.com/
wANNOVAR, http://wannovar.wglab.org/

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