

Supplementary material

Gain- and loss-of-function *GRIA3* variants lead to distinct neurodevelopmental phenotypes

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Supplementary methods

Beta-lactamase (β -lac) reporter assay

HEK293 cells were transiently transfected with cDNA encoding β -lac-tagged WT GluA3 or GluA3 variants according to the protocol in Stenum-Berg et al.⁹⁴. Non-transfected cells were used as a negative control for surface β -lac activity. AMPAR inhibitors (100 μ M CNQX, 100 μ M perampanel, and 1 μ M NASPM) were added to the medium during transfection to protect against potential excitotoxicity. After 48 hrs, the transfected cells were rinsed twice with PBSCM (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄, 0.1 mM CaCl₂, 0.5 mM MgCl₂, pH 7.4). For analysis in HEK293T cells, transfected cells were cultured in poly-D-lysine coated clear-bottom 96-well plates for two days. Cells were washed twice in PBSCM, followed by adding nitrocefin to a final concentration of 50 μ M in a total volume of 100 μ L per well. To define total expression levels, cells were rinsed with PBSCM, lysed by adding 50 μ L H₂O, and incubated for 30 minutes before adding 50 μ L 2xPBSCM containing 100 μ M nitrocefin. Immediately following nitrocefin addition, plates were placed in a microplate reader (Safire2, Tecan, Maennedorf, Switzerland), and well absorbance at 486 nm was recorded every minute for 60 minutes at ~30 °C. For each well, the absorbance at 486 nm (Abs486 nm) was plotted as a function of time using GraphPad Prism v6.01 (GraphPad Software, San Diego, CA, USA), and the rate of nitrocefin conversion per minute was determined by linear regression analysis of the slope of the curve in the linear range.

Protein modeling

We modeled the flip isoform of the human homomeric GluA3 receptor in the closed *apo* conformation using the X-ray crystal structure of the homomeric rat GluA2 receptor in complex with the competitive antagonist ZK (PDB ID: 5L1B). Alignment of the rat GluA2 template structures to the GluA3 sequence was aided by sequence alignment of the human and rat

iGluA3 and GluA2 sequences (Supplementary Figure S1). Homology modeling was performed using the modeling software MODELLER (Sali and Blundell, 1993). A total of 100 models were created, and the best model were chosen based on Discrete Optimized Protein Energy (DOPE) scores calculated in MODELLER.

Supplementary results

Phenotypic analysis of patient cohort with disease-causing *GRIA3* variants

We were able to collect detailed clinical information for 25 patients from 23 unrelated families carrying disease-causing *GRIA3* variants. The median age at study inclusion was 9.5 years (range 5 months – 39 years of life) (Table 1). Of the 25 patients, clinical data for seven were previously published^{26,29,33,34}, but we were able to collect additional clinical data. Notably, 21 patients were unrelated, while M5/M6 and M11/M12 were brothers. Three girls (F5, F7, and F9) passed away prematurely at median age 2 years (range 5 months – 3½ years). Patient F5 died of respiratory failure during prolonged and treatment-resistant status epilepticus. Patient F7 died at home due to hyperekplexia with apnea. Patient F9 was intubated due to pneumonia. Taking her off ventilation was impossible, and she died of respiratory failure. All patients exhibited cognitive and global developmental delay ranging from borderline to profound. While a single patient carrying the p.(Gln371Argfs*6 variant had a borderline low-normal IQ at 78, the remainder of developmental outcomes were either mildly to moderately (1/25), moderately, (9/25), severely (9/25), severe to profoundly (2/25) or profoundly (4/25) affected. Although eight patients remain nonverbal, most can communicate using simple words (5/25) or short sentences (8/25). First words were commonly acquired between 13-36 months of life. A single patient (F2) had mild dyslexia but otherwise spoke almost fluently. There was no data on the present verbal abilities of two patients (F4 and F10). While 10 patients remain non-ambulant, most walked independently (14/25) or with support (1/25). Of the 25 patients, 15 displayed behavioral issues that were variable. Stereotypies or further unspecified autistic features were present in five and six patients, respectively. Non-epileptic staring spells were reported in three patients. Nine patients had a low anger threshold with tantrums and occasional aggressive outbursts.

Twelve patients had seizures with median seizure onset around 12 months of age (range 1 day to 27 years). Seizure types included focal motor (6/12), myoclonic (5/12), unknown onset motor (4/12), (atypical) absence (2/12), focal impaired awareness (1/12), and generalized tonic-clonic (1/12) or atonic (1/12) seizures. We classified epilepsies based on seizure types; focal onset epilepsy (4/12) was most common, followed by generalized (2/12) and combined focal and generalized epilepsy (1/12). Five patients were reported to have unknown onset seizures that co-occurred with focal or generalized onset seizures in two patients. Eight patients were classified as having developmental and epileptic encephalopathy (DEE), 13 were classified as having developmental encephalopathy without seizures, and four had intellectual disability with epilepsy. None of the patients could be further classified into a specific epilepsy syndrome. Drug-resistant epilepsy with ongoing seizures was seen in 7/8 patients with DEE.

Interestingly, perampanel, a negative allosteric modulator of AMPAR, was only prescribed in a single patient with seizures and congenital muscle hypertonia: Patient F4, carrying the p.(Ser663Pro) variant which leads to a severe GoF, had rigidity and stimulus-sensitive and spontaneous non-epileptic myoclonus in the neonatal period and developed focal tonic seizures at 2 months of life. Levetiracetam was introduced with partial seizure control. Numerous generalized onset myoclonic seizures started at four months, leading to the introduction of perampanel (2mg nightly; 0.37mg/kg/day) that led to a significant reduction in tonic and myoclonic seizure frequency as well as the duration of tonic seizures. The hypertonicity also decreased, and she began making significant developmental progress. Perampanel was eventually increased to 5 mg twice daily by age 18 months of life, but myoclonic seizures persisted, particularly upon awakening. Parents reported a substantial decrease in the frequency and severity of myoclonic seizures with perampanel use. An electroencephalogram repeated after perampanel initiation showed fewer interictal discharges

during wakefulness and sleep, but frequent myoclonic seizures remained. She currently has no rigidity and minimal spasticity.

Limb muscular hypotonia was reported in 12/25 patients. Muscular hypertonia and spasticity were reported in ten and six patients, respectively. Movement disorders were reported in 15/25, with hyperekplexia or non-epileptic erratic myoclonus being the most prevalent features (8/25), followed by ataxia (6/25).

MRI brain results were available for 18 patients and were reported to be normal in 14. In the remaining 4 patients, there were only nonspecific findings, such as abnormal corpus callosum (1/4), focal cortical dysplasia (1/4), cerebellar vermis hypoplasia (1/4), and signal abnormality in the periventricular white matter (1/4) (Table 1 and Supplementary Table S7).

Supplementary Table S1. Genetic characteristics of GRIA3 missense variants in patients with neurodevelopmental disorders.

Variant	Gender	Origin	cDNA	ChrX:g.	CADD ^a	SIFT ^b	PolyPhen2 ^c	gnomAD frequency, % ^d	Source
p.(Phe53Leu)	I M	De novo	c.159T>G	g.122319733T>G	23.7	Deleterious (0.04)	Prob. Damaging (0.98)	0	ClinVar
p.(Arg169Gln)	I M	Maternal	c.506G>A	g.122387391G>A	29.8	Tolerated (0.18)	Prob. Damaging (0.99)	0	This study
p.(Ala248Val)	I F	De novo	c.743C>T	g.122488807C>T	22.2	Deleterious (0.02)	Prob. Neutral (0.074)	0	Allen et al.
p.(Pro302Ser)	I M	De novo	c.904C>T	g.122528972C>T	24.1	Tolerated (0.41)	Prob. Damaging (0.99)	0	This study
p.(Ile314Thr)	M	De novo	c.941T>C	g.122532515T>C	23.8	Deleterious (0.01)	Poss. Damaging (0.87)	0	This study
p.(Gln371Argfs*6)	I M	Maternal	c.1112_1116del	g.122536876_122536880del	ND	ND	ND	0	This study
p.(Gln371His)	F	De novo	c.1113A>C	g.122536877A>C	23.8	Deleterious (0.02)	Poss Damaging (0.64)	0	This study
p.(Phe408Leu)	I M	Maternal	c.1222T>C	g.123403448T>C	2.4	Tolerated (0.73)	Neutral (0.00)	0	This study
p.(Arg450Gln)	I M	Maternal	c.1349G>A	g.122538614G>A	22.9	Deleterious (0.02)	Neutral (0.20)	0.0029	Wu et al.
p.(Gly492Ser)	I M	Maternal	c.1474G>A	g.122538739G>A	28.6	Deleterious (0.00)	Prob. Damaging (1.00)	0	This study
p.(Arg501Thr)	I F	De novo	c.1502G>C	g.122551254G>C	32.0	Deleterious (0.02)	Prob. Damaging (0.97)	0	Powis et al.
p.(Ser531Cys)	I F	NA	c.1592C>G	g.122551344C>G	27.3	Deleterious (0.00)	Prob. Damaging (0.95)	0	This study
p.(Ile564Thr)	I M	De novo	c.1691T>C	g.122551443T>C	29.1	Deleterious (0.00)	Prob. Damaging (0.92)	0	This study
p.(Ala615Val)	I M	De novo	c.1844C>T	g.122551596C>T	28.5	Deleterious (0.00)	Prob. Damaging (0.98)	0	Hamanaka, et al.
p.(Met617Thr)	NA	NA	c.1850T>C	g.122551602T>C	25.7	Deleterious (0.00)	Prob. Damaging (0.98)	0	ClinVar
p.(Gly630Arg)	4 M	Maternal	c.1888G>C	g.122561802G>C	28.9	Deleterious (0.00)	Prob. Damaging (1.00)	0	This study; Wu, et al.
p.(Arg631Ser)	I M	Maternal	c.1891C>A	g.122561805C>A	26.3	Deleterious (0.00)	Prob. Damaging (1.00)	0	Wu, et al.
p.(Ser647Phe)	I F	NA	c.1940C>T	g.122561854C>T	29.8	Deleterious (0.00)	Prob. Damaging (0.99)	0	Clinvar
p.(Ala653Thr)	2 M	Maternal	c.1957G>A	g.122561871G>A	28.8	Deleterious (0.00)	Prob. Damaging (0.98)	0	Davies, et al.
p.(Ala654Pro)	I F	De novo	c.1960G>C	g.122561874G>C	28.9	Deleterious (0.00)	Prob. Damaging (0.99)	0	This study
p.(Ala654Val)	I F	De novo	c.1961C>T	g.122561875C>T	27.9	Deleterious (0.00)	Prob. Damaging (0.92)	0	This study
p.(Ala654Thr)	I F	De novo	c.1960G>A	g.122561874G>A	28.9	Deleterious (0.00)	Prob. Damaging (0.95)	0	This study
p.(Phe655Ser)	2 M	Maternal/mosaic	c.1964T>C	g.122561878T>C	29.8	Deleterious (0.00)	Prob. Damaging (1.00)	0	Cherot, et al.
p.(Arg660Ser)	I F	NA	c.1980G>C	g.122561894G>C	24.9	Deleterious (0.00)	Prob. Damaging (1.00)	0	ClinVar
p.(Arg660Thr)	I F	De novo	c.1979G>C	g.122561893G>C	27.9	Deleterious (0.00)	Prob. Damaging (0.99)	0	Sun, et al.
p.(Ser663Pro)	I F	De novo	c.1987T>C	g.122561901T>C	27.9	Tolerated (0.28)	Prob. Damaging (0.96)	0	This study
p.(Ile665Thr)	I F	NA	c.1994T>C	g.122561908T>C	26.9	Deleterious (0.00)	Prob. Damaging (1.00)	0	This study
p.(Lys701Glu)	I F	Maternal	c.2101A>G	g.122598740A>G	26.4	Deleterious (0.00)	Prob. Damaging (0.99)	0	This study
p.(Met706Thr)	I M	Maternal	c.2117T>C	g.122598756T>C	25.4	Deleterious (0.00)	Prob. Damaging (0.97)	0	Wu, et al.
p.(Gly721Arg)	4 M	Maternal	c.2161G>A	g.122598800G>A	29.2	Deleterious (0.00)	Prob. Damaging (1.00)	0	Hu, et al.
p.(Gly730Ala)	NA	NA	c.2189G>C	g.122598828G>C	27.3	Deleterious (0.00)	Prob. Damaging (0.98)	0	ClinVar
p.(Met740Thr)	NA	NA	c.2219T>C	g.122598858T>C	23.3	Tolerated (0.14)	Prob. Damaging (0.97)	0	ClinVar
p.(Leu774Ser)	I M	NA	c.2321T>C	g.122598960T>C	27.0	Deleterious (0.00)	Prob. Damaging (1.00)	0	ClinVar

p.(Thr776Met)	1 M	NA	c.2327C>T	g.122613916C>T	21.8	Deleterious (0.01)	Prob. Damaging (1.00)	0	Turro, et al.
p.(Glu787Gly)	1 M	Maternal	c.2359G>A	g.122599559G>A	31.0	Deleterious (0.00)	Prob. Damaging (0.92)	0	Rinaldi, et al.
p.(Glu787Lys)	1 F + 2 M	Maternal/ De novo	c.2360A>G	g.122599560A>G	29.8	Deleterious (0.00)	Prob. Damaging (0.99)	0	Trivisano, et al.
p.(Trp799Leu)	1 F	De novo	c.2396G>T	g.122599596G>T	29.8	Deleterious (0.00)	Prob. Damaging (0.99)	0	This study
p.(Gly803Glu)	2 F + 1 M	De novo	c.2408G>A ; c.2408G>A	g.122599608G>A ; g.122613997G>A	28.9	Deleterious (0.01)	Prob. Damaging (1.00)	0	This study, Yang, et al.
p.(Gly803Ala)	1 M	Maternal	c.2408G>C	g.122599608G>C	26.9	Tolerated (0.44)	Prob. Damaging (1.00)	0	This study
p.(Thr816Ile)	1 F	NA	c.2447C>T	g.122616657C>T	28.1	Deleterious (0.00)	Prob. Damaging (0.98)	0	Yang, et al.
p.(Gly826Asp)	4 M	Maternal	c.2477G>A	g.122616687G>A	28.2	Deleterious (0.00)	Prob. Damaging (0.96)	0	Pieard, et al.
p.(Gly833Arg)	1 M	Maternal	c.2497G>A	g.122616707G>A	31.0	Deleterious (0.00)	Prob. Damaging (1.00)	0	Wu, et al.
p.(Arg856Gly)	1 M	NA	c.2566C>G	g.122616776C>G	23.9	Deleterious (0.00)	Prob. Damaging (0.86)	0	This study
p.(Leu859Phe)	1 M	Maternal	c.2575C>T	g.122616785C>T	24.2	Deleterious (0.00)	Neutral (0.19)	0	This study

The table shows genetic information of *GRIA3* variants identified in NDD patients included in the study, the resultant change in amino acid, the genomic DNA nucleotide change (GRCh37), and the site of the variants in cDNA sequence for the GluA3 subunit protein (NM_000828.4) along with calculated scores for the predicted deleteriousness of the variants from various programs that predicts whether an amino acid substitution affects protein function. ^aSorting Intolerant From Tolerant (SIFT)⁹⁵; ^bCombined annotation-dependent depletion (CADD) scores⁹⁶; ^cPolymorphism Phenotyping v2 (PolyPhen2)⁹⁶. F, female; M, male; NA, not available; ND: not determined.

Supplementary Table S2. Current amplitudes of homomeric GluA3 and heteromeric GluA2/A3 receptors

Variant	Homomeric GluA3				Heteromeric GluA2/A3			
	Glu current, nA [95% CI]	n	Glu +CTZ current, nA [95% CI]	n	Glu current, nA [95% CI]	n	Glu + CTZ current, nA [95% CI]	n
WT	93 [57-129]	175	4232 [3240-5224]	132	628 [478-779]	135	6128 [478-7661]	106
p.(Phe53Leu)	110 [83-137]	46	2378 [1111-3646]	9	ND		ND	
p.(Arg169Gln)	69 [39-99]	15	2989 [1421-4558]	8	ND		ND	
p.(Ala248Val)	141 [86-196]	20	4224 [-606-9054]	5	ND		ND	
p.(Pro302Ser)	118 [70-166]	21	6079 [3755-8403]	11	ND		ND	
p.(Ile314Thr)	122 [68-176]	14	7071 [1128-13010]	8	ND		ND	
p.(Gln371Argfs*6)	0 [0-0]***	15	0 [0-0]***	15	ND		ND	
p.(Gln371His)	166 [4-327]	22	3633 [-896-8161]	8	ND		ND	
p.(Phe408Leu)	227 [72-382]	23	4421 [1526-7317]	9	ND		ND	
p.(Arg450Gln)	12 [5-18]***	60	742 [510,9-972,3]***	44	224 [119-330]***	21	8698 [119-12420]	32
p.(Gly492Ser)	0 [0-0]***	22	0 [0-0]***	22	109 [82-135]***	57	1422 [82-2260]	34
p.(Arg501Thr)	66 [43-88]	89	3199 [1743-4655]	41	1592 [730-2453]	17	15240 [730-20490]	6
p.(Ser531Cys)	1 [1-1]***	31	2580 [1011-4149]	22	629 [195-1063]	17	4543 [195-7253]	21
p.(Ile564Thr)	154 [71-237]	29	4454 [2089-6818]	12	ND		ND	
p.(Ala615Val)	294 [221-366]***	31	9328 [7146-11510]*	15	3210 [2470-3949]***	22	16940 [2470-20740]	14
p.(Met617Thr)	28 [4-52]***	16	462 [332,6-590,9]***	16	81 [40-122]***	24	3606 [40-4555]	26
p.(Gly630Arg)	0 [0-1]***	16	1 [0-1]***	19	7 [4-11]***	16	54 [4-78]	33
p.(Arg631Ser)	1045 [585-1506]***	49	2075 [1069-3081]	23	1090 [894-1286]*	26	1320 [894-1558]	28
p.(Ser647Phe)	48 [38-58]	58	1357 [797-1918]***	12	2593 [1998-3187]***	54	48060 [1998-58180]	20
p.(Ala653Thr)	28 [20-35]***	38	831 [487,6-1174]***	10	133 [41-226]***	22	7780 [41-11350]	11
p.(Ala654Prol)	ND		ND		101 [46-155]***	20	56 [46-81]	36
p.(Ala654Val)	5377 [3356-7397]***	27	6591 [3974-9209]	27	1728 [891-2566]	24	2354 [891-3851]	12
p.(Ala654Thr)	649 [431-867]***	28	741 [312-1169]***	13	3019 [2637-3401]	13	3403 [2637-3837]	13
p.(Phe655Ser)	1 [0-1]***	33	68 [45-91]***	66	185 [96-274]	45	4497 [96-6925]	19
p.(Arg660Ser)	90 [54-126]	84	2309 [1757-2862]*	35	1244 [911-1577]	60	12080 [911-15520]	26
p.(Arg660Thr)	592 [430-753]***	43	1898 [1433-2364]**	26	1665 [724-2606]	25	6615 [724-9916]	13
p.(Ser663Pro)	1102 [751-1453]***	55	7333 [5601-9064]***	32	2463 [1923-3003]	22	10260 [1923-13660]	11
p.(Ile665Thr)	31 [19-43]***	25	489 [280,1-698,7]***	17	496 [399-593]	18	12740 [399-16030]	9
p.(Lys701Glu)	687 [397-977]***	38	4211 [2538-5884]	15	2402 [1559-3244]	57	3924 [1559-6558]	16
p.(Met706Thr)	1 [0-1]***	14	1 [0-1]***	14	162 [117-206]	16	3147 [117-5575]	6
p.(Gly721Arg)	0 [0-1]***	14	1 [0-1]***	14	1 [0-1]	18	10 [-1-12]	10
p.(Gly730Ala)	67 [54-80]	68	3047 [2357-3737]	33	225 [105-346]	25	3765 [105-6368]	7
p.(Met740Thr)	90 [70-110]	81	5343 [4211-6475]	39	439 [210-669]	26	4528 [210-7754]	10
p.(Leu774Ser)	5 [4-7]***	33	1430 [1097-1763]***	58	376 [311-443]	80	14650 [311-18570]	35
p.(Thr776Met)	30 [21-39]*	53	2214 [1494-2934]	23	259 [173-346]	21	3644 [173-5845]	10

p.(Glu787Lys)	22 [1-42]*	21	90 [43-137]***	10	137 [-81-355]	18	144 [-81-227]	10
p.(Glu787Gly)	0 [0-0]***	10	0 [0-0]***	10	72 [42-102]	25	7565 [42-10920]	17
p.(Trp799Leu)	34 [20-48]	32	4387 [2323-6452]	17	461 [360-561]	29	12110 [360-15070]	9
p.(Gly803Glu)	95 [69-120]	53	9128 [6431-11820]*	47	1205 [490-1921]	43	7157 [490-9924]	9
p.(Gly803Ala)	80 [0-160]	35	1739 [1139-2339]**	25	310 [202-418]	26	3750 [202-4978]	15
p.(Thr816Ile)	25 [11-38]*	18	1152 [382-1921]***	9	85 [41-129]	19	2035 [41-3186]	9
p.(Gly826Asp)	6 [2-9]***	21	1197 [848-1545]***	33	505 [370-641]	32	14870 [370-18270]	18
p.(Gly833Arg)	0 [0-0]***	14	9 [7-12]***	13	13 [9-17]	17	118 [9-149]	17
p.(Arg856Gly)	68 [39-97]	23	5953 [3253-8654]	13	ND		ND	
p.(Leu859Phe)	114 [63-165]	20	4739 [1768-7710]	9	232 [86-378]	19	6903 [86-10260]	9

The table shows a summary of the maximal Glu-evoked current amplitudes of WT and *GRIA3*-variant containing GluA3 and GluA2/A3 receptors at -40 mV membrane potential measured by TEVC recordings and expressed as mean with 95% CI. *N* represents the number of individual cells subjected to experiments. Currents were recorded over a -20 to -100 mV range of holding potential, and transformed to the current amplitude at -40 mV using the equation $I_{-40} = I_x * \gamma_x$, where *X* is the holding potential and γ_x is the conversion factor calculated from IV relationship of the Glu-evoked current at homomeric GluA3 receptors as γ_{-100} 0.07, γ_{-90} 0.11, γ_{-80} = 0.15, γ_{-70} = 0.22, γ_{-60} 0.36, γ_{-50} 0.54, γ_{-40} 1.00, γ_{-30} 1.94, γ_{-20} 4.08, and at heteromeric GluA2/A3 receptors as γ_{-100} 0.44, γ_{-90} 0.47, γ_{-80} 0.51, γ_{-70} 0.58, γ_{-60} 0.67, γ_{-50} 0.80, γ_{-40} 1.00, γ_{-30} 1.34, γ_{-20} 2.20. **P* < .05, ***P* < .01, ****P* < .001 compared to wild-type by one way ANOVA, with Dunnett's multiple comparisons test. ND, not determined as the homomer variant receptor showed neutral phenotype, except in the case of p.(Ala654Pro), due to constitutive activity.

Supplementary Table S3. Summary of electrophysiological data for homomeric GluA3 receptors

Variant	EC50, μM [95% CI]	N	EC50 with CTZ, μM [95% CI]	N	$I_{\text{GLU}}/I_{\text{GLU}+\text{CTZ}}$, % ^a	N	$I_{\text{KA}}/I_{\text{GLU}}$, % ^b	N	NASPM inhibition, %	N
WT	29 [27-33]	94	87 [78-99]	38	1.9[1.6-2.2]	71	18 [16-19]	67	88 [84-91]	45
p.Phe53Leu	28 [26-30]	17	NR		4.4[3.6-5.1]	9	18 [14-23]	8	87 [63-111]	4
p.Arg169Gln	33 [29-37]	18	NR		1.4[1.1-1.8]	18	19 [17-22]	15	87 [80-93]	12
p.Ala248Val	40 [33-46]	9	NR		3.5[3.3-3.8]	5	23 [15-30]	11	97 [96-98]	7
p.Pro302Ser	34 [29-40]	7	NR		1.7[1.3-2.1]	13	17 [13-21]	7	95 [90-101]	7
p.Ile314Thr	36 [29-42]	9	NR		2.1[1.4-2.7]	7	23 [16-29]	13	82 [72-92]	6
p.Gln371Argfs*6	ND		ND		ND		ND		ND	
p.Gln371His	77 [68-86]*	8	NR		1.3[1.0-1.6]	13	21 [15-26]	13	83 [76-90]	8
p.Phe408Leu	48 [42-53]	8	NR		1.8[0.7-2.9]	12	20 [17-23]	11	87 [75-99]	6
p.Arg450Gln	ND		85 [78-93]	22	1.0[0.8-1.2]	32	19 [17-21]	33	87 [83-92]	27
p.Gly492Ser	ND		ND		ND		ND		ND	3
p.Arg501Thr	12 [11-14]**	22	NR		1.4[0.8-1.9]	34	19 [16-21]	34	86 [81-92]	33
p.Ser531Cys	ND		1.5 [1.0-2.0]***	11	0.5[0.3-0.8]*	15	22 [18-26]	15	96 [81-110]	4
p.Ile564Thr	143 [110-170]***	7	NR		3.4[2.7-4.0]	15	16 [11-21]	13	84 [79-89]	20
p.Ala615Val	25 [23-28]	14	NR		32[26-40]***	14	33 [26-40]**	14	29 [18-41]***	17
p.Met617Thr	ND		443 [388-506]***	8	1.2[1.0-1.4]	14	8 [5-10]**	16	64 [55-73]***	14
p.Gly630Arg	ND		ND		ND		ND		ND	
p.Arg631Ser	10 [8.2-11]***	12	NR		101[93-109]***	22	54 [50-57]***	20	119 [113-126]	18
p.Ser647Phe	29 [27-31]	26	NR		2.3[1.6-3.0]	20	31 [28-35]***	25	89 [82-96]	14
p.Ala653Thr	39 [36-43]***	11	NR		1.7[1.2-2.3]	10	2 [1-2]***	10	77 [70-84]	11
p.Ala654Pro	ND		ND		75[63-87]***	14	ND	14	301 [266-336]***	12
p.Ala654Val	3.5 [3.2-3.8]	10	NR		88[84-92]***	27	56 [47-65]***	27	92 [89-95]	26
p.Ala654Thr	1.64 [0.79-2.7]	12	NR		55[49-60]***	13	55 [49-61]***	13	97 [95-99]	13
p.Phe655Ser	ND		1258 [1198-1320]***	31	11.4[9.8-13.1]***	26	11 [10-13]**	26	95 [81-109]	5
p.Arg660Ser	10 [9-11]***	20	NR		1.6[1.4-1.8]	35	45 [42-48]***	32	89 [84-94]	14
p.Arg660Thr	15 [14-17]***	20	NR		28[25-31]***	24	43 [39-47]***	21	94 [89-99]	12
p.Ser663Pro	61 [52-72]	17	NR		16.5[12.8-20.3]***	29	27 [23-31]**	23	89 [86-93]	33
p.Ile665Thr	20 [17-239]	8	NR		6.3[5.1-7.6]	9	9 [7-10]*	8	84 [69-98]	7
p.Lys701Glu	56 [52-59]	21	NR		8.4[6.6-10.1]*	15	8 [7-10]**	15	94 [90-98]	15
p.Met706Thr	ND		ND		ND		ND		ND	
p.Gly721Arg	ND		ND		ND		ND		ND	
p.Gly730Ala	30 [29-31]	10	NR		3.1[2.5-3.8]	33	17 [15-19]	34	82 [76-88]	29
p.Met740Thr	22 [20-24]	11	NR		1.4[1.0-1.8]	42	12 [10-13]	26	78 [72-84]	22
p.Leu774Ser	ND		212 [185-242]	10	0.5[0.3-0.6]***	28	21 [19-23]	30	84 [75-92]	14
p.Thr776Met	217 [199-236]	10	NR		0.7[0.5-0.8]*	22	21 [18-23]	19	97 [92-101]	16
p.Glu787Gly	ND		ND		ND		ND		ND	

p.Glu787Lys	ND		51 [38-68]	6	ND		ND		ND	
p.Trp799Leu	0.57 [0.27-0.87]	8	NR		0.1[0.0-0.1]***	18	108 [95-122]***	17	86 [77-95]	6
p.Gly803Glu	0.87 [0.67-0.92]	10	NR		1.6[1.1-2.0]	37	48 [43-54]***	35	97 [90-105]	13
p.Gly803Ala	1.3 [1.1-1.5]	10	NR		0.3[0.2-0.4]***	21	25 [22-27]*	24	80 [74-85]	10
p.Thr816Ile	190 [178-201]	10	NR		0.3[0.2-0.4]*	8	20 [12-28]	9	91 [85-97]	9
p.Gly826Asp	187 [180-194]	8	NR		0.6[0.4-0.7]*	14	3 [2-4]***	21	38 [19-58]***	9
p.Gly833Arg	ND		ND		ND		ND		ND	
p.Arg856Gly	60 [48-72]	12	NR		0.9[0.7-1.1]	12	13 [10-16]	12	67 [58-76]	7
p.Leu859Phe	6.2 [4.4-6.4]	7	NR		2.2[1.2-3.2]	8	11 [8-14]	9	96 [93-99]	9

Data were generated by TEVC recordings with -20 to -100 mV of holding potential, and were expressed as mean \pm 95 CI. *n* indicates number of individual cells. ^a The ratio of current responses to consecutive applications of Glu in absence and presence of CTZ. ^b The ratio of current responses to consecutive applications of kainate and glutamate (KA/Glu) determined in the presence of CTZ. **P* < .05, ***P* < .01, ****P* < .001 compared to WT by one-way ANOVA, with Dunnett's multiple comparisons test. *ND*, not determined, due to no or small current, except in the case of p.(Ala654Pro), due to constitutive activity. *NR*, not relevant.

Supplementary Table S4. Summary of TEVC data for heteromeric GluA2/A3 receptors

Variant	EC50, μM [95% CI]	N	EC50 with CTZ, μM [95% CI]	N	$I_{\text{GLU}}/I_{\text{GLU+CTZ}}$, %	N	$I_{\text{KA}}/I_{\text{GLU}}$, % ^b	N
WT	23 [26-30]	55	78 [74-83]	15	7.5 [6.6-8.3]	80	32 [31-34]	73
p.Arg450Gln	44 [42-47]	12	NR		4.4 [2.7-6.1]	22	23 [19-28]	22
p.Gly492Ser	36 [34-38]	24	NR		6.9 [5.7-8.1]	25	31 [29-33]	25
p.Ser531Cys	ND		24 [21-28]	8	4.5 [3.4-5.5]	11	31 [28-33]	11
p.Ile564Thr	40 [37-44]	8	NR		6.8 [5.1-8.5]	10	27 [22-33]	10
p.Ala615Val	31 [30-32]	7	NR		19 [17-21]	14	54 [51-58]	14
p.Met617Thr	59 [48-73]***	13	NR		4.0 [1.6-6.4]	17	17 [16-19]	17
p.Gly630Arg	ND		51 [48-55]***	11	9.2 [5.7-12.6]	12	19 [16-23]	12
p.Arg631Ser	25 [23-27]	10	NR		76 [70-81]***	22	54 [50-57]	20
p.Ser647Phe	35 [32-38]	21	NR		6.9 [5.3-8.5]	15	37 [34-41]	15
p.Ala653Thr	52 [50-55]***	11	NR		2.4 [0.9-3.9]**	11	4.7 [3.9-5.4]	8
p.Ala654Prol	ND		ND		130 [113-148]***	8	65 [63-68]	8
p.Ala654Val	20 [19-21]**	10	NR		70 [65-76]***	11	34 [33-36]	11
p.Ala654Thr	17 [15-19]**	13	NR		89 [85-93]***	13	51 [48-54]	12
p.Phe655Ser	30[26-35]	23	NR		1.0 [0.8-1.2]		11 [10-13]	18
p.Arg660Ser	17 [16-18]**	27	NR		20 [14-25]**	23	53 [46-59]	22
p.Arg660Thr	25 [21-28]	13	NR		43 [37-49]***	13	44 [42-47]	10
p.Ser663Pro	31 [30-32]	11	NR		26 [23-29]**	10	49 [47-52]	11
p.Ile665Thr	48 [46-52]***	9	NR		4.3 [3.2-5.4]	9	14 [11-17]	9
p.Lys701Glu	33 [31-35]	25	NR		27 [21-32]***	16	31 [26-36]	14
p.Met706Thr	55 [52-58]**	7	NR		7.0 [4.1-10.0]	6	15 [13-17]	6
p.Gly721Arg	ND		ND		ND		ND	
p.Leu774Ser	42 [40-44]***	30	NR		2.7 [2.2-3.1]**	35	25 [23-28]	35
p.Thr776Met	33 [30-35]**	15	NR		4.2 [2.7-5.6]	24	29 [26-31]	25
p.Glu787Gly	25 [24-30]***	15	NR		0.5 [0.2-0.8]***	17	32 [27-37]	17
p.Glu787Lys	72 [63-83]	1	NR		6.4 [4.2-8.7]	8	121 [106-137]	8
p.Trp799Leu	1.3 [1.1-1.5]***	16	NR		5.4 [4.5-6.3]	9	56 [52-61]	9
p.Gly803Glu	15 [12-17]***	15	NR		4.6 [3.6-5.6]	15	44 [39-49]	15
p.Gly803Ala	8.9 [6.8-11]***	10	NR		5.8 [4.7-7.0]	15	42 [38-46]	15
p.Thr816Ile	35 [30-39]	10	NR		1.9 [1.5-2.4]	9	27 [23-31]	9
p.Gly826Asp	29 [27-31]	14	NR		2.7 [1.5-2.3]	18	29 [27-31]	18
p.Gly833Arg	ND		69 [66-74]	8	14.4 [6.3-22.4]*	8	23 [17-29]	5

Electrophysiological data were generated by TEVC recordings with -20 to -100 mV of holding potential, and were expressed as mean with 95% CI. *N* is the number of experiments on individual cells. ^a The ratio of current responses to saturating concentration of glutamate (GLU) in absence (I_{GLU}) and presence ($I_{\text{GLU+CTZ}}$) of block of desensitization by cyclothiazide (CTZ). ^b The ratio of current responses to consecutive applications of kainate and glutamate ($I_{\text{KA}}/I_{\text{GLU}}$) determined in the presence of CTZ. * $P < .05$, ** $P < .01$, *** $P < .001$ compared to WT by one way ANOVA (analysis of variance), with Dunnett's multiple comparisons test. ND, not determined, due to no or small current. NR, not relevant.

Supplementary Table S5. Summary of the kinetic parameters of wild-type and variant GluA3 and GluA2/A3 receptors

Variant	Isoform	τ_{desens} , ms	N	I_{ss} , %	N	τ_{deact} , ms	N
WT	Flip	5.1 ± 0.3	12	1.1 ± 0.1	16	2.1 ± 0.2	9
+ A2	Flip	6.9 ± 0.7	11	2.9 ± 0.1	11	2.4 ± 0.2	10
WT	Flop	1.6 ± 0.1	10	0.82 ± 0.30	9	1.2 ± 0.1	12
+ A2	Flop	3.1 ± 0.3	9	0.66 ± 0.12	13	1.4 ± 0.1	12
p.(Gly492Ser)	Flip	ND		ND		ND	
+ A2	Flip	ND		ND		ND	
p.(Gly630Arg)	Flip	ND		ND		ND	
+ A2	Flip	ND		ND		ND	
p.(Ala654Prol)	Flip	ND		100 ± 1	4	>20	4
+ A2	Flip	ND		100 ± 1	4	>40	4
p.(Ala654Val)	Flip	6.5 ± 1.5	8	61 ± 2 ^{***}	9	9.8 ± 1.0 ^{***}	10
+ A2	Flip	8.6 ± 0.2	9	70 ± 4 ^{***}	9	8.2 ± 1.3 ^{***}	12
p.(Ala654Thr)	Flip	ND		82 ± 3 ^{***}	9	5.3 ± 1.3 ^{***}	6
+ A2	Flip	26 ± 2.0 ^{***}	22	18 ± 2 ^{***}	22	1.9 ± 0.1 ^{***}	16
p.(Phe655Ser)	Flip	ND		ND		ND	
+ A2	Flip	ND		ND		ND	
p.(Arg660Thr)	Flip	12.9 ± 1.4 ^o		28.1 ± 5.2 ^o		6.3 ± 0.3 ^o	
+ A2	Flip	12.8 ± 0.9 ^o		12.6 ± 2.6 ^o		1.5 ± 0.2 ^o	
p.(Ser663Pro)	Flip	10.4 ± 1.0 ^{***}	10	7.0 ± 1.0 ^{***}	10	2.5 ± 0.2	8
+ A2	Flip	10.7 ± 0.8 ^{***}	11	5.6 ± 0.8 ^{***}	11	2.0 ± 0.1	16
p.(Ile665Thr)	Flip	ND		ND		ND	
+ A2	Flip	4.8 ± 0.3	10	2.2 ± 0.4 [*]	9	1.9 ± 0.1	8
p.(Lys701Glu)	Flip	13 ± 1.1 ^{***}	8	6.8 ± 1.5 ^{***}	11	4.5 ± 0.4 ^{***}	12
+ A2	Flip	15 ± 1.0 ^{***}	10	6.2 ± 0.8 ^{***}	11	4.5 ± 0.3 ^{***}	12
p.(Glu787Gly)	Flop	ND		ND		ND	
+ A2	Flop	ND		ND		ND	
p.(Glu787Lys)	Flop	ND		ND		ND	
+ A2	Flop	ND		ND		ND	
p.(Trp799Leu)	Flop	ND		ND		ND	
+ A2	Flop	2.9 ± 0.1	16	0.7 ± 0.2 ^{***}	11	1.3 ± 0.1	12
p.(Gly803Glu)	Flip	7.3 ± 0.9	9	2.2 ± 0.6	9	5.0 ± 0.4 ^{**}	10
+ A2	Flip	9.1 ± 0.6	11	1.6 ± 0.2	11	4.7 ± 0.4	11
p.(Gly803Ala)	Flip	5.6 ± 0.6	10	2.1 ± 0.4	10	4.6 ± 0.6 [*]	11
+ A2	Flip	9.6 ± 1.0	10	1.8 ± 0.3	10	3.5 ± 0.3	10

Data were generated by patch-clamp recordings with -40 to -80 mV of holding potential, and were expressed as mean ± SEM (n). ^oFrom Sun, et al. (2021). ND, not determined, due to no or small current, except for p.(Ala654Pro), p.(Ala654Thr), and p.(Trp799Leu), which showed current waveforms that did not permit reliable fitting of the desensitization and deactivation phases due to profoundly slowed (p.(Ala654Pro) and p.(Ala654Thr)) or accelerated (p.(Trp799Leu)) kinetics.

Supplementary Table S7. Extended clinical and genetic information of patients with neurodevelopmental disorders carrying a loss- or gain-of-function variants in *GRIA3*, part A.

Family	1	2	3	4	4	5	6	7	8	9
ID number	M1	M2	M3	M4 (brother of M5)	M5 (brother of M4)	M6	F10	M7	M8	M9
Age	18 y	39 y	15y	14 y	12 y	5.5 y	28 y	21 y	4.5 y	6 y
Previously published	No	No	No	Yes (PMID 35697757)	Yes (PMID 35697757)	No	No	Yes (PMID 34731330)	Yes (PMID 32977175)	No
DNA change (NM_007325.5)	c.1112_1116del	c.1474G>A	c.1888G>C	c.1888G>C	c.1888G>A	c.1888G>C	c.1994T>C	c.2360A>G	c.2359G>A	c.2359G>A
Protein change	p.(Gln371Argfs*6)	p.(Gly492Ser)	p.(Gly630Arg)	p.(Gly630Arg)	p.(Gly630Arg)	p.(Gly630Arg)	p.(Ile665Thr)	p.(Glu787Gly)	p.(Glu787Lys)	p.(Glu787Lys)
Genetic test	TRIO WES	NGS panel for ID genes	WES	Quadruple-WES	Quadruple-WES	WES	WGS	WES	WES	WES
Inheritance	Maternally inherited	Maternally inherited	Maternally inherited	Maternally inherited	Maternally inherited	De novo	Unknown	Maternally inherited	Maternally inherited	Maternally inherited
Functional effect of variant	Severe loss-of-function	Severe loss-of-function	Severe loss-of-function	Severe loss-of-function	Severe loss-of-function	Severe loss-of-function	Severe loss-of-function	Severe loss-of-function	Severe loss-of-function	Severe loss-of-function
Degree of global developmental delay	Borderline (IQ 78)	Moderate	Severe	Mild-moderate	Moderate	Moderate	Moderate	Severe	Profound	Severe
Age of sitting/walking	8 mo/ 13 mo	NA / 24 mo	1 y / 27 mo	NA / 30 mo	1 y / 30 mo	2 y / 3 y	NA / 4 y	NA / 13 mo	NA / NA	Non-ambulant
Present motor skills	Independent walking	Independent walking, running	Independent walking	Independent walking, running	Independent walking, running	Independent walking, stairs with support, long distances wheelchair dependent	Non-ambulant	Independent walking	Non-ambulant, unable to hold head or sit	Non-ambulant, unable to hold head or sit
First words	3 y	NA	2.5 y	2.5 y	27 m	NA	NA	1 y	Nonverbal	Nonverbal
Present verbal ability	Short sentences, dysarthria	Short sentences	Simple words	Unknown but able to read and write	Short sentences	Short sentences, dysarthria	NA	Short sentences, dysarthria	Nonverbal	Nonverbal
Neurologic findings	Dysarthria	Muscular hypotonia, hyporeflexia	Muscular hypotonia, hyporeflexia	Muscular hypotonia	Muscular hypotonia	Muscular hypotonia, hyporeflexia	Brisk tendon reflex, limb spasticity, gait dyspraxia	Muscular hypotonia, hyporeflexia	Muscular hypotonia, hyporeflexia	Muscular hypotonia, hyporeflexia
Movement disorders	No	No	No	No	No	Ataxia	Dystonic head posturing	Ataxia, intention tremor, non-epileptic erratic myoclonus	No	No
Epilepsy diagnosis	No	No	Yes	No	No	No	No	Yes	Yes	Yes
Age at onset of seizure	NR	NR	Yes (17 mo)	NR	NR	NR	NR	Yes (29 mo)	Yes (16 mo)	Yes (1 y)

Seizure type	NR	NR	FTS, FBTCs	NR	NR	NR	NR	FCS, generalized onset My, My status epilepticus.	AtypAS with eyelid My, My, non-convulsive SE	Generalized My, tonic, and TCS
Treatment resistant seizures	NR	NR	Yes	NR	NR	NR	NR	Until 14 y, then seizure-free on ESM and CLZ	Yes	Yes
EEG findings	NA	NA	NA	NA	NA	NA	NA	Interictal EEG with poor background activity, bilateral frontal epileptic discharges regions; ictal discharges consisted of bilateral frontal or diffuse spike and wave	EEG showed poor background with multifocal discharges	Generalized slow wave with spike or spike wave discharges
Brain MRI	Normal (1 y)	NA	Normal (2 y)	NA	NA	Signal abnormality in the periventricular white matter on the right, suggestive of gliosis and (1.2 y and 2.5 y)	NA	Cerebellar vermis hypoplasia, focal cortical dysplasia	Normal (1 y)	Normal (17 mo)
Behavioural issues	No	NA	Stereotypies, anxiety, AO, ASD	Echolalia, AO, ASD	Stereotypies, echolalia, AO, ASD	Echolalia, difficulties with self-regulation, self-harming, stereotypies, reduced attention span.	ASD	AO	No	No
Sleep disorders	No	No	Yes (long wakefulness)	Yes (long wakefulness, nocturnal awakening)	Yes (long wakefulness, nocturnal awakening)	Yes (nocturnal awakening)	Yes (long wakefulness)	NA	Yes (long wakefulness)	No

Supplementary Table S7. Extended clinical and genetic overview of patients with neurodevelopmental disorders carrying a loss- or gain-of-function variants in *GRIA3*, part B.

Family	I0	I1	I2	I2	I3	I4	I5	I6	I7	I8
ID number	F11	M10	M11 (brother of M12)	M12 (brother of M11)	F9	M13	M14	F1	F2	F3
Age	13 y	12 y	38 y	33 y	2 y (deceased due to a pneumonia and respiratory failure)	7.5 y	9.5 y	30 y	8 y	5y
Previously published	No	No	Yes (PMID 29016847)	Yes (PMID 29016847)	No	No	No	No	No	No
DNA change (NM_007325.5)	c.2359G>A	c.1964T>C	c.1957G>A	c.1957G>A	c.2396G>T	c.2408G>C	c.2408G>C	c.2408 G>A	c.2408 G>A	c.2101A>G
Protein change	p.(Glu787Lys)	p.(Phe655Ser)	p.(Ala653Thr)	p.(Ala653Thr)	p.(Trp799Leu)	p.(Gly803Ala)	p.(Gly803Glu)	p.(Gly803Glu)	p.(Gly803Glu)	p.(Lys701Glu)
Genetic test	WES	WES	WGS	WGS	WES	WES	Trio WES	WES	Trio WES	WES
Inheritance	De novo	Maternally inherited	Maternally inherited	Maternally inherited	De novo	Maternally inherited	De novo	De novo	De novo	De novo
Functional effect of variant	Severe loss-of-function	Severe loss-of-function	Mild loss-of-function	Mild loss-of-function	Mild loss-of-function	Mild gain-of-function	Mild gain-of-function	Mild gain-of-function	Mild gain-of-function	Mild gain-of-function
Degree of global developmental delay	Severe	Severe	Severe	Severe	Severe/profound	Severe/profound	Severe	Profound	Moderate	Moderate
Age of sitting/walking	6 mo / 1.4 y	Non-ambulant	NA / 3.5 y	3 y / 7 y	Non-ambulant	9 mo / 27 mo	Non-ambulant	18 mo / 24 mo (aided)	6 mo / 16 mo	2 y / 18 mo with walker
Present motor skills	Independent walking	Non-ambulant. Able to roll, unable to sit	Independent walking and running (balance problems)	Independent walking and running (balance problems)	No motor skills, unable to hold head or sit	Independent walking	No motor skills, unable to hold head or sit	Wheelchair bound after operation for scoliosis	Independent walking, running, swimming	Sits unsupported, walks w/ walker, not running or climbing stairs
First words	NA	Nonverbal	13 m	14 mo	Nonverbal	3 y	Nonverbal	1 y	18 mo	1 y
Present verbal ability	Short sentences	Nonverbal	Simple words	Simple words	Nonverbal	Simple words	Nonverbal	Short sentences	Almost fluent, mild dyslexia	Simple words
Neurologic findings	Muscular hypotonia, hyporeflexia	Muscular hypotonia, hyporeflexia	Muscular hypotonia, hyporeflexia	Muscular hypotonia, hyporeflexia	Muscular hypertonia	Brisk deep tendon reflexes	Muscular hypertonia, limb spasticity, brisk deep tendon reflexes	Muscular hypertonia, limb spasticity, brisk deep tendon reflexes	Muscular hypertonia	Muscular hypertonia, limb spasticity, brisk deep tendon reflexes
Movement disorders	Ataxia	No	Ataxia, tremor	Ataxia, tremor	Excessive startle (less present over time), tremor, dyskinesia, dystonia (onset 5 mo).	Ataxia	Dystonia, hyperekplexia, chorea	NA	Intention tremor	Hyperekplexia, reflex non-epileptic myoclonia during neonatal age

Epilepsy diagnosis	Yes	No	No	No	Yes	Yes	Yes	Yes	No	No
Age at onset of seizure	Yes (3 y)	NR	NR	NR	Yes (9 mo)	Yes (1 y)	Yes (1 d)	Yes (27 y)	NR	NR
Seizure type	Absences, unknown onset My, unknown onset BTCS. SE	NR	NR	NR	Focal clonic seizures (face and limb)	Focal migrating seizures. Unknown onset brief (few seconds) impaired awareness seizures	Unknown onset tonic and atonic seizures	Unknown onset TCS	NR	NR
Treatment resistant seizures	Yes	NR	NR	NR	Seizure free on levetiracetam and oxcarbazapine)	Yes	Yes	No (only 2 seizures, treatment not initiated)	NR	NR
EEG findings	NA	NA	NA	EEG showed poor background with multifocal discharges (occasional spikes and separate slow waves mainly right anterior quadrant)	At 9 months EEG showed poor background with multifocal sharp waves	NA	Day 1: Burst suppression pattern. Day 7: no burst suppression, discontinuous asynchronous EEG with multifocal sharp waves. 1 mo: abnormal awake and asleep EEG due to bilateral posterior spikes indicating regions of cortical irritability, diffuse beta background activity	NA	NA	NA
Brain MRI	Normal (12 y)	NA	Normal (19 y)	NA	Normal (5 mo)	Normal (4 y)	Normal (2 m)	NA	Normal (5 mo)	Rathke's cyst (2 y)
Behavioural issues	Stereotypies, hyperactivity, AO	Stereotypies, non-epileptic staring spells	Stereotypies, non-epileptic staring spells	Stereotypies, non-epileptic staring spells	No, but difficult to assess due to age.	Stereotypies, anxiety, AO, ASD	NA	OCD	Mild anxiety	No
Sleep disorders	Yes (long wakefulness)	NA	Yes (long wakefulness)	Yes (long wakefulness)	Yes	Yes (long wakefulness)	No	No	No	No

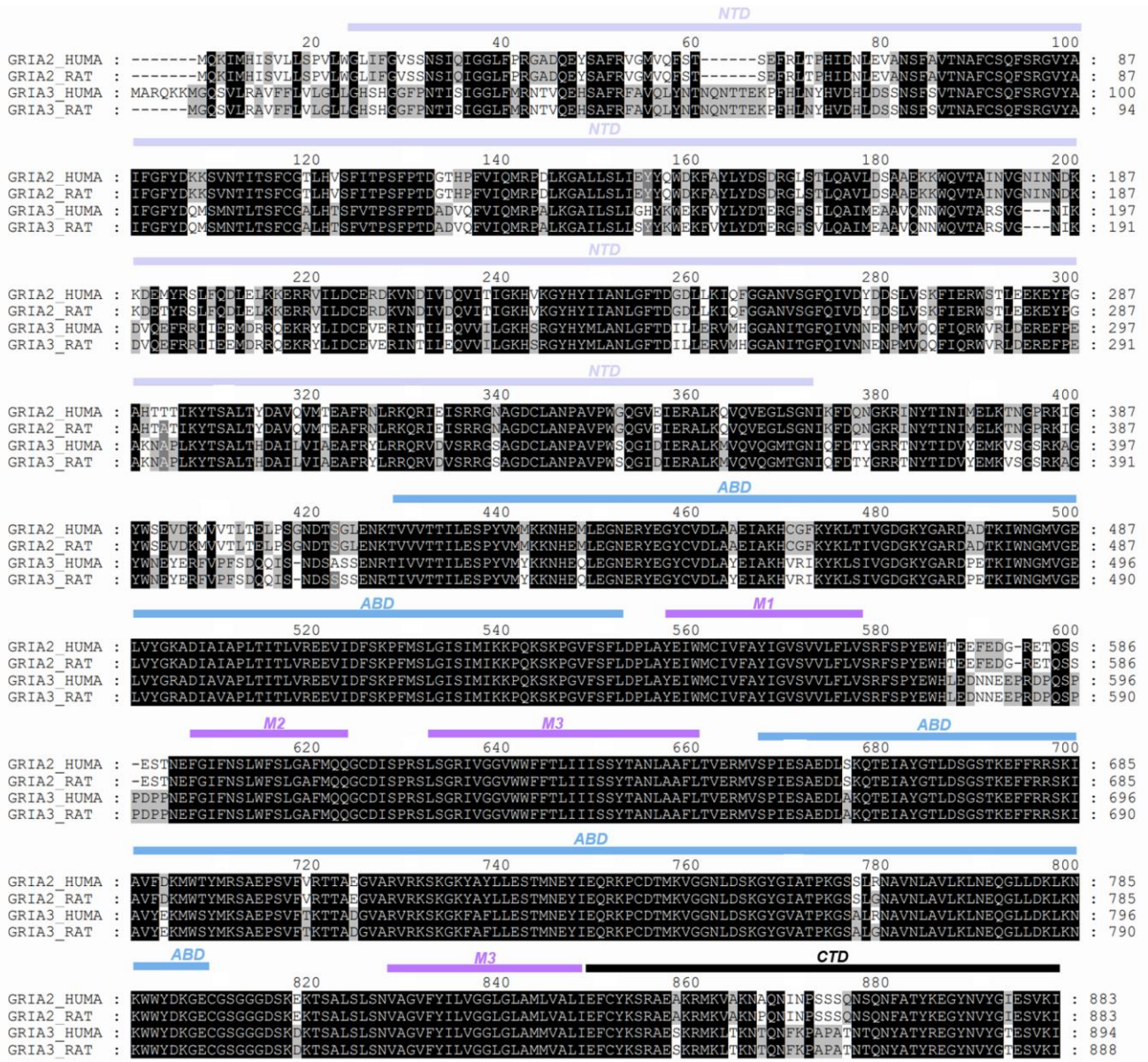
Supplementary Table S7. Extended clinical and genetic overview of patients with neurodevelopmental disorders carrying a loss- or gain-of-function variants in GRIA3, part C.

Family	19	20	21	22	23
ID number	F4	F5	F6	F7	F8
Age	9 mo	3.5 y (deceased)	9 y	5 mo (deceased at home due to apnea with hyperekplexia)	5 y
Previously published	No	No	No	No	Yes (PMID 34161333)
DNA change (NM_007325.5)	c.1987T>C	c.1961C>T	c.1960G>C	c.1960G>A	c.1979G>C
Protein change	p.(Ser663Pro)	p.(Ala654Val)	p.(Ala654Pro)	p.(Ala654Thr)	p.(Arg660Thr)
Genetic test	WES	WES	WES	WES	WGS
Inheritance	De novo	De novo	De novo	De novo	De novo
Functional effect of variant	Severe gain-of-function	Severe gain-of-function	Severe gain-of-function	Severe gain-of-function	Severe gain-of-function
Degree of global developmental delay	Moderate	Profound	Moderate. Development largely plateaued.	Severe	Profound
Age of sitting/walking	NA /NA	Non-ambulant	> 1 y / 26 mo	NA / NA	Non-ambulant
Present motor skills	Can briefly hold her head, sits supported	Non-ambulant, unable to hold head or sit	Independent walking (poor balance, narrow gate). Runs unsteady.	No motor skills, unable to hold head or sit	Non-ambulant, unable to hold head or sit
First words	NA	Nonverbal	3 y	Nonverbal	Nonverbal
Present verbal ability	NA	Nonverbal	Can say long sentences but mostly they are short and repetitive	Nonverbal	Nonverbal
Neurologic findings	Muscular hypertonia	Muscular hypertonia, brisk deep tendon reflexes	Muscular hypertonia, limb spasticity, brisk deep tendon reflexes	Muscular hypertonia	Muscular hypertonia, limb spasticity, brisk deep tendon reflexes
Movement disorders	Hyperekplexia, reflex non-epileptic myoclonia	Hyperekplexia, reflex non-epileptic myoclonia, tremor	NA	Hyperekplexia, non-epileptic myoclonus	Hyperekplexia, reflex non-epileptic myoclonia

Epilepsy diagnosis	Yes	Yes	No	No	Yes
Age at onset of seizure	Yes (2 mo)	Yes (10 d)	NR	NR	Yes (1 d)
Seizure type	FTS, generalized My	FTS. FBTCS with apnea	NR	NR	Unknown onset My and unknown onset TCS
Treatment resistant seizures	No (Initiation of PER at 5 mo of life; no FTS after initiation and reduced frequency and severity of My)	Yes (partial seizure free on a combination of LMT, VPA and CLZ)	NR	NR	Yes
EEG findings	NA	NA	NA	NA	NA
Brain MRI	Normal (2 mo)	Normal (1 mo)	Normal (3 y)	Normal (8 d)	Hypoplastic CC (neonatal age)
Behavioural issues	NA	Crying spells, irritability	AO, ASD, reduced attention span	NA	NA
Sleep disorders	No	Yes	Yes (long wakefulness, nocturnal awakening)	NA	NA

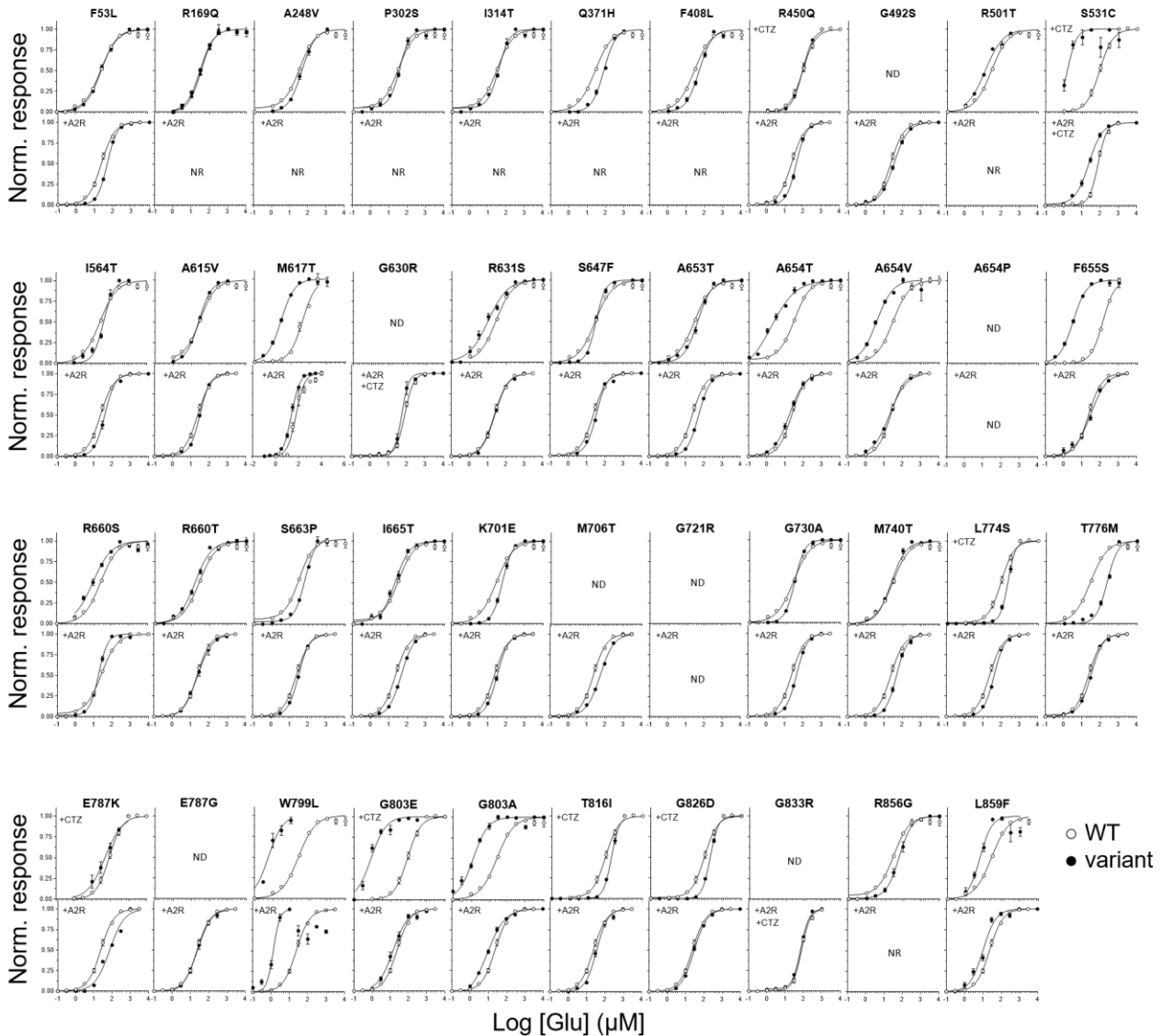
The table shows clinical and genetic information for patients M1-M14 and F1-F11. Abbreviations used are: Abbreviations: AO = aggressive outburst; ASD = autism spectrum disorder; AtypAS = atypical absences; BTCS = bilateral tonic clonic seizures; CC = corpus callosum; CLZ = clonazepam; ESM; ethosuximide; FBTCS: focal to bilateral tonic clonic seizures; FCS = focal clonic seizure; ID = intellectual disability; LMT = lamotrigine; LEV = levetiracetam; mo = months; MRI = magnetic resonance imaging; My = myoclonia; NA: not available; NGS = next generation sequencing; NR = not relevant; OCD = obsessive compulsive disorder; OXC = oxcarbazepine; PER = perampenal; SE = status epilepticus; VPA = valproate; y = years; WES = whole exome sequencing; WGS = whole genome sequencing.

Supplementary Figure S1. Amino acid sequence alignment of human and rat GluA3 and GluA2 subunits used for homology modelling of the human GluA3 receptor



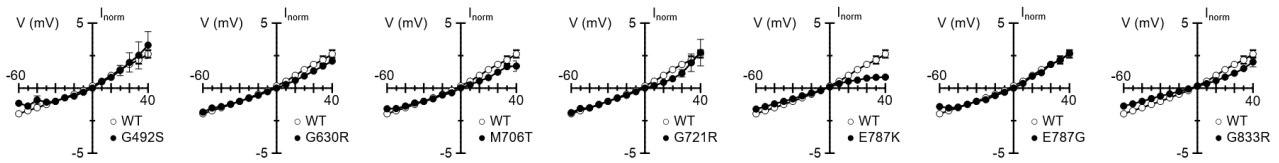
Protein sequence alignment of human GluA2 (GRIA2_HUMA), rat GluA3 (GRIA2_RAT), human GluA3 (GRIA3_HUMA), and rat GluA3 (GRIA3_RAT) subunits. Dashes indicate gaps in the alignment. Shading of residues in the alignment is applied to provide overview of the level and pattern of residue conservation in the subunit sequences: *Black* color indicate residue identity among all subunits, *dark gray* color indicate identity among three subunits, and *light gray* indicate identity among two subunits. Note that no shading can also indicate identity among two subunits. The location of the NTD segment (*light purple*), the ABD segments (*blue*), and the transmembrane regions M1 to M4 (*magenta*) are depicted on top of the alignment.

Supplementary Figure S2. Concentration-response curves for glutamate



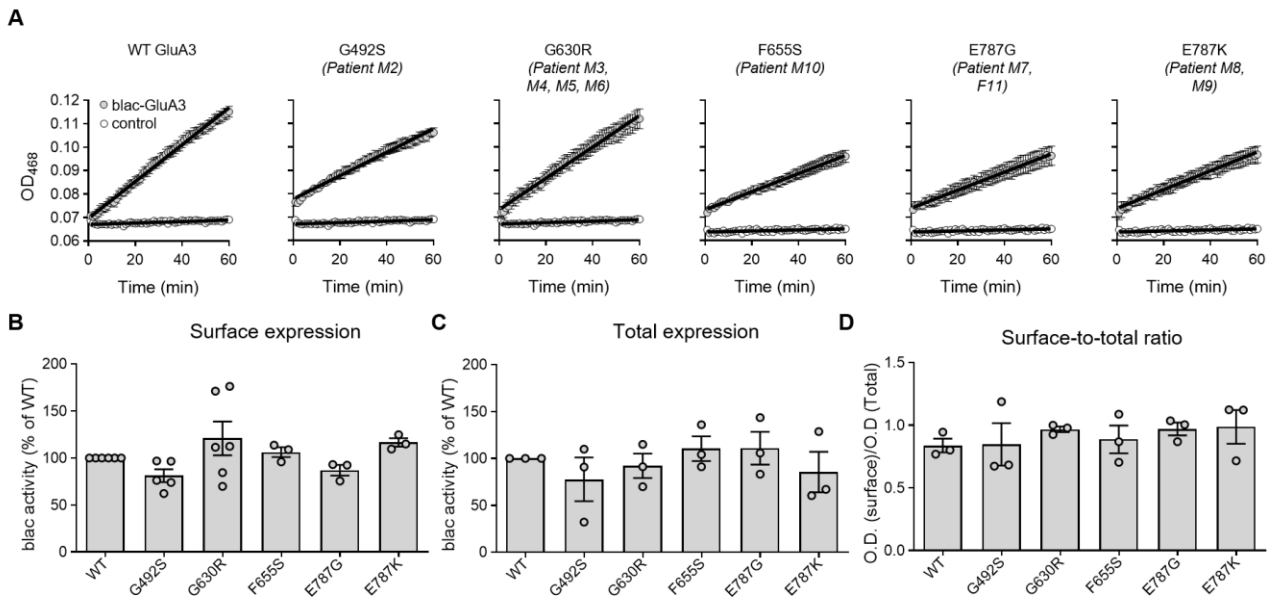
Composite concentration-response curves for Glu for homomeric GluA3 and heteromeric GluA2/A3 receptors for WT and receptors containing the variant-encoded genetic changes in GluA3. Each graph shows curves for the indicated mutant (●) and WT (○). CTZ (100 μ M) was included for homomeric GluA3 receptors with the R450Q (Arg450*), S531C (Ser531Cys), M611T (Met611Thr), F649S (Phe649Ser), E787K (Glu787Lys), W799L (Trp799Leu), T810I (Thr810Ile), and G826D (Gly826Asp), and G833R (Gly833Arg) variants and for heteromeric GluA2/A3 receptors with the R450Q (Arg450*), S531C (Ser531Cys), G630R (Gly630Arg), and G833R (Gly833Arg) variants to increase response amplitude. Data in all composite concentration-response curves are mean \pm SEM.

Supplementary Figure S3. Current-voltage relationships of heteromeric GluA2/A3 with loss-of-function variants



IV relationships of Glu-evoked currents from oocytes expressing heteromeric WT GluA2/A3 (*white circles*) and variant-containing GluA2/A3 (*black circles*) receptors. The current amplitude at the different holding potentials is normalized to the current at -40 mV. Data points represent the mean from 3 to 6 oocytes. Error bars indicate the SEM and are shown when larger than the symbol size. In all panels, variants are labelled with single-letter amino acid codes.

Supplementary Figure S4. *GRIA3* variants with complete LoF effects have intact receptor cell surface expression



(A) Representative plots of nitrocefin conversion measured as absorbance at 465 nm (OD₄₆₅) as a function of time are shown for live intact HEK293 cells grown in 96-well plates and transfected with WT blac-tagged GluA3 or blac-tagged GluA3 subunit (blac-GluA3, *gray circles*) with genetic changes encoded by *GRIA3* missense variants that showed little or no current response to Glu in the fast-application patch-clamp experiments. Nitrocefin conversion rates for non-transfected cells (control; *white circles*) measured in parallel are shown. Solid lines show linear regression fits to determine the nitrocefin conversion rates that represent blac-activity. Each data point represents the mean \pm SEM from 4 wells. **(B-C)** Summary of expression levels of blac-GluA3 carrying the variant-encoded genetic changes relative to WT blac-GluA3. The rates were measured at intact cells that represent surface expression levels **(B)**, and at lysed cells that represent total expression **(C)**. The nitrocefin conversion rates were averaged ($n = 3-5$ independent experiments) and are shown as percentages of the rates for WT determined in parallel. Data are expressed as the mean \pm SEM from the indicated individual experiments. **(D)** Ratio of surface-to-total expression.