Functional analysis of a *de novo* GRIN2A missense mutation associated with early-onset epileptic encephalopathy

Hongjie Yuan¹, Kasper B. Hansen¹, Jing Zhang¹, Tyler Mark Pierson²,³, Thomas C. Markello³, Karin V. Fuentes Fajardo³, Conisha M. Holloman³, Gretchen Golas³, David R. Adams³, Cornelius F. Boerkoel³, William A. Gahl³ & Stephen F. Traynelis¹

NMDA receptors (NMDARs), ligand-gated ion channels, play important roles in various neurological disorders, including epilepsy. Here we show the functional analysis of a *de novo* missense mutation (L812M) in a gene encoding NMDAR subunit GluN2A (*GRIN2A*). The mutation, identified in a patient with early-onset epileptic encephalopathy and profound developmental delay, is located in the linker region between the ligand-binding and transmembrane domains. Electrophysiological recordings revealed that the mutation enhances agonist potency, decreases sensitivity to negative modulators including magnesium, protons and zinc, prolongs the synaptic response time course and increases single-channel open probability. The functional changes of this amino acid apply to all other NMDAR subunits, suggesting an important role of this residue on the function of NMDARs. Taken together, these data suggest that the L812M mutation causes overactivation of NMDARs and drives neuronal hyperexcitability. We hypothesize that this mechanism underlies the patient’s epileptic phenotype as well as cerebral atrophy.
NMDA receptors (NMDARs) are ligand-gated ion channels that mediate excitatory neurotransmission in the brain. NMDARs are activated following the binding of glutamate and glycine to the GluN1 and GluN2 subunits, respectively. Simultaneous binding of both co-agonists opens a cation-selective pore that results in both depolarization and an increase in the intracellular Ca$^{2+}$ concentration. The GluN1 subunit is expressed universally throughout the brain, whereas four different GluN2 subtypes (A–D) have varying expression patterns. The GluN2A and GluN2C subunits are expressed mainly after birth, whereas the expression of GluN2B and GluN2D subunits is highest prenatally and is diminished postnatally in most (but not all) brain regions. NMDARs play important roles in neuronal development, synaptic plasticity, learning and memory, as well as in a wide range of pathological conditions, including epilepsy, schizophrenia, Huntington and Parkinson diseases.

Recently, pathogenic mutations in genes encoding NMDAR subunits (GRIN1, GRIN2A and GRIN2B) have been identified in human neurodevelopmental phenotypes, such as, epilepsy, developmental delay or intellectual disability. Since many early-onset epileptic encephalopathies are due to mutations in ion channels or receptors, we screened for de novo mutations in ion channel or receptors; we identified a de novo GRIN2A mutation: NCBI nucleotide accession number NM_001134407.1: c.2434C>A (p.Leu812Met, hereafter referred to as L812M), absent in his unaffected sibling as well as in his unaffected parents. The Leu812 residue is highly conserved across the NMDA receptor family.

Results

Identification of GRIN2A L812M mutation. A six-year-old boy was admitted to the NIH Undiagnosed Diseases Program with a history of intractable infantile-onset epilepsy and profound global developmental delay with no attainment of any milestones, not even head control. At presentation he was having daily, generalized seizures that had been refractory to multiple anticonvulsants including lacosamide, rufinamide and valproic acid. Brain MRI at 6 years of age showed diffuse cerebral parenchymal volume loss and a thin corpus callosum disproportionate to the loss of cortical grey matter. There were no dysmorphic features and ophthalmological anomalies. Overall, his clinical history and features were consistent with an early-onset epileptic encephalopathy.

Since many early-onset epileptic encephalopathies are due to de novo mutations in ion channels or receptors, we screened for de novo potentially dominant variants and paired potentially recessive variants with whole-exome sequencing. The only remarkable deleterious variant identified was a heterogeneous, de novo GRIN2A mutation: NCBI nucleotide accession number NM_001134407.1: c.2434C>A (p.Leu812Met, hereafter referred to as L812M), absent in his unaffected siblings as well as in his unaffected parents. The Leu812 residue is highly conserved across vertebrate species and GluN1 and all GluN2 NMDAR subunits (Fig. 1a), indicating a possible critical role in NMDAR function.

GluN2A–Leu812 resides in the linker region between the lower portion of the agonist-binding domain (S2) and the transmembrane domain (M4) (Fig. 1a,b). Using the AMPA receptor structure as a guide, this residue is predicted to reside close enough (within 5 Å) to interact with two potential gating regions in the GluN1 subunit, the M3 transmembrane helix and the pre-M1 helix (Fig. 1c). Multiple lines of evidence suggest that a conserved motif (SYTANLAAF) and Gly-814 in the M1 transmembrane helix controls gating.

Moreover, in AMPA receptors the pre-M1 helix lies in van der Waals contact with the gate and has been suggested to act as a cuff around gating elements that may stabilize the closed state. Interestingly, this site is also a locus for allosteric regulation in NMDA, AMPA and kainate receptors. Our working hypothesis is that the mutation...
GluN2A–L812M enhances agonist potency. To investigate whether the mutation GluN2A–L812M influences NMDAR function, site-directed mutagenesis was used to introduce L812M into cDNA encoding the human GluN2A gene product (hereafter hGluN2A). We subsequently expressed wild-type (WT) and mutant hGluN2A with human GluN1 (hGluN1) in Xenopus oocytes and evaluated the concentration–effect curves for glutamate and glycine using a two-electrode voltage-clamp (TEVC). The mutation L812M increased the glutamate potency, as measured by reduction in the half-maximally effective concentration of agonist (EC$_{50}$) from 3.5 to 0.41 µM (Fig. 2a, left; Table 1). Glycine potency was similarly increased, with the EC$_{50}$ decreasing from 1.1 µM in WT to 0.14 µM in mutant receptors (Fig. 2a, right);
In contrast to the results for agonist potency, a single copy of Mg

decreases in potency were observed at other holding potentials (for example, Table 1) shows that more current flows in the single copy of Mg

mutant receptors at the negative holding potentials (for example, Table 1) shows that more current flows in the single copy of Mg

decrease in EC50 to 0.20 for WT receptors at the holding potential of

NATURE COMMUNICATIONS | DOI: 10.1038/ncomms4251 | www.nature.com/naturecommunications

ARTICLE NATURE COMMUNICATIONS | DOI: 10.1038/ncomms4251

Table 1 | Summary of pharmacological data for L812M.

|                | Di-heteromeric receptors | 2A-L812M | Tri-heteromeric receptors | 2A/2A | L812M/2A | L812M/L812M |
|----------------|--------------------------|----------|---------------------------|-------|----------|-------------|
| Glu EC50 µM (n) | WT 2A | 3.5 ± 0.21 (15) | 0.41 ± 0.04 (24)* | 4.0 ± 0.20 (10) | 1.0 ± 0.07 (10)† | 0.39 ± 0.04 (11)† |
| Gly, EC50 µM (n) | 1.1 ± 0.05 (24) | 0.14 ± 0.01 (39)* | 0.72 ± 0.04 (10) | 0.20 ± 0.01 (8)† | 0.06 ± 0.007 (8)† |
| Mg2+ ||| | 33 ± 1.3% (7) | 60 ± 6.8% (8)* | 35 ± 6.8% (8) | 55 ± 5.0% (13)† | 57 ± 2.8% (8)† |
| Mg2+, IC50 µM (n)|| | 17 ± 12 (19) | 43 ± 2.9 (21)* | 15 ± 2.6 (7) | 38 ± 2.8 (8)† | 41 ± 2.8 (8)† |
| Proton, IC50 pH (n)|| | 6.9 (6-10) | 6.4 (6-11) | 6.9 (6-18) | 6.5 (6-9) | 6.4 (6-7) |
| Zinc, IC50 nM (n)|| | 77 ± 7.8 (7) | NE | 91 ± 3.9 (9) | NE | NE |
| % inhibition by zinc || | 49 ± 3.2% (7) | 6.1 ± 1.7% (6)* | 34 ± 1.8% (14) | 7.8 ± 0.9% (10)† | 4.8 ± 1.1% (6)† |

NE, no effect.
The data were expressed as mean ± s.e.m. (n).
*P<0.05 compared with WT 2A, unpaired t-test.
†P<0.05 compared with 2A/2A: one-way ANOVA, Tukey post hoc.
| Percentage current remaining in the presence of 0.3 mM MgCl2 at | wt of 47 m s (n = 11; Fig. 3a, left; Table 2). The data were expressed as mean ± s.e.m. (n).
|                | 60 mV | 74% (10) | 0.007 (8)† |
|                | 80 mV | 4.8% (8)† |

Table 1). These data showed that the L812M mutation enhanced the potency of glutamate and glycine by eightfold, which will allow the mutant receptor to be activated by much lower concentration of the agonists.

Since the L812M mutation of this patient is heterozygous and the tetrameric NMDAR complex contains two GluN2 subunits, we anticipate that some receptors in the patient will contain only a single copy of the mutation. We therefore employed a strategy to control GluN2 trafficking and thus the subunit composition of cell surface NMDARs. We used GluN2A with heterodimeric coiled-coil domains appended to their C termini along with a short d lysine ER retention/retrieval signal comprising KKTN coiled-coil domains appended to their C termini along with a short d lysine ER retention/retrieval signal comprising KKTN (see Methods). We made a similar pair of constructs harbouring the GluN2A–L812M mutation, which allowed us to express surface receptors with 0, 1 or 2 copies of the mutant GluN2A. We subsequently explored the pharmacology and biophysical properties of these tri-heteromeric receptors. The receptors that contained a single copy of the mutant subunit GluN2A–L812M (referred to as L812M/2A) showed an intermediate increase in glutamate potency (4-fold shift in EC50 to 1.0 µM from 4.0 µM for 2A/2A; Fig. 2b, left; Table 1) and glycine potency (3.6-fold decrease in EC50 to 0.20 µM from 0.72 µM for 2A/2A; Fig. 2b, right; Table 1). Thus, NMDAR activation was enhanced at lower extracellular concentrations of the agonist even for receptors with a single copy of the mutation.

GluN2A–L812M attenuates voltage-dependent Mg2+ block. Voltage-dependent channel block of the NMDAR by extracellular Mg2+ is an important feature of NMDARs, rendering current flow voltage-dependent29,30. Two experiments were performed to test whether the GluN2A–L812M alters Mg2+ inhibition. Evaluation of the current–voltage relationship (Fig. 2c; Table 1) shows that nearly twice as much current (60 versus 33% for WT) will flow in the mutant compared to WT receptors at negative holding potentials (−30 mV). The concentration–response curves for Mg2+ block (Fig. 2e; Table 1) show that this mutation increased the Mg2+ IC50 value to 43 µM from 17 µM for WT receptors at the holding potential of −60 mV; similar decreases in potency were observed at other holding potentials (WT versus L812M IC50 was 455 versus 820 µM at −20 mV, 74 versus 174 µM at −40 mV and 4.7 versus 11 µM at −80 mV). In contrast to the results for agonist potency, a single copy of L812M (L812M/2A) produced a dominant reduction in the Mg2+ inhibition. The current–voltage relationship (Fig. 2d; Table 1) shows that more current flows in the single copy of mutant receptors at the negative holding potentials (for example, 55 versus 35% of 2A/2A at −30 mV). A single copy of GluN2A–L812M (Table 1) increased Mg2+ IC50 to 38 µM from 15 µM for 2A/2A at the holding potential of −60 mV.

As Mg2+ block occurs deep within the ion channel pore and involves the reentrant pore loops that also control Ca2+ permeability31–33, we tested whether the L812M mutation alters the calcium permeability. Measurement of the reversal potentials for WT hGluN2A, L812M and WT hGluN2D receptors (as a positive control) in bi-ionic conditions (Ca2+ or Cs+) allows the determination of relative Ca2+ permeability. The data in Supplementary Fig. 1 show that L812M has no detectable effect on the relative Ca2+ permeability.

GluN2A–L812M diminishes negative allosteric regulation. Endogenous proton and zinc can inhibit NMDAR function in the central nervous system3, and these endogenous ions likely serve as important regulatory mechanisms that limit NMDAR activation. We therefore tested whether the L812M mutation can influence regulation by extracellular zinc or protons. GluN2A–L812M reduced the proton sensitivity, with mutant receptors showing an IC50 corresponding to pH 6.4, compared with pH 6.9 for WT (Fig. 2f, left; Table 1). The mutation also virtually eliminated high-affinity zinc inhibition for GluN2A–L812M, compared with WT receptors that were inhibited with an IC50 of 77 nM (Fig. 2f, right; Table 1). Interestingly, a single copy of L812M (L812M/2A) produced a dominant reduction in proton sensitivity with an IC50 of pH 6.5, compared with pH 6.9 for 2A/2A (Table 1). The single copy of the mutation also produced a dominant decrease in the sensitivity of the receptor to 300 nM extracellular zinc (2A/2A: 34%; L812M/2A: 7.8%; L812M/L812M: 4.8%; Table 1).

GluN2A–L812M prolongs deactivation time course. We next assessed the effect of L812M mutation on the response time course of NMDARs. The deactivation rate following removal of glutamate from NMDARs is thought to control the time course of the NMDAR component of the excitatory postsynaptic current (EPSC)34. To evaluate whether the mutation altered the deactivation time course, the current response following glutamate removal was measured using a rapid solution exchange system in whole-cell patch-clamp recordings of WT GluN2A or mutant L812M co-expressed with GluN1 in HEK 293 cells. The deactivation time course of WT 2A following rapid removal of glutamate was fitted by two exponential components with a weighted tau (τw) of 47 ms (n = 11; Fig. 3a, left; Table 2). The mutant L812M receptors significantly prolonged the glutamate deactivation time course, which was fitted by two exponential components with a τw of 443 ms (n = 10; Fig. 3a, left; Table 2).
We also briefly moved the cell into the agonist solution for 3–5 ms (brief application) to mimic synaptic events in the brain. As with the long pulse of agonist, the mutant receptors deactivated with a slower time course compared with WT NMDARs (τ_W 425 versus 44 ms for WT; Fig. 3a, right). The deactivation time course following glycine removal was measured, and could be fitted by two exponential components with a τ_W of 63 ms (n = 5; Fig. 3b; Table 2). The mutant GluN2A–L812M receptors showed a slower glycine deactivation time course, which was fitted by two exponential components with a τ_W of 691 ms (n = 5; Fig. 3b; Table 2).

We additionally assessed the time course of deactivation following removal of glutamate or glycine for receptors containing one copy of GluN2A–L812M. As expected, the glutamate and glycine deactivation time constants of receptors with two copies of mutant GluN2A (L812M/L812M) and receptors with no mutation (2A/2A) were comparable to those determined for GluN2A–L812M and WT GluN2A, respectively (Fig. 3c,d; Table 2). However, the receptors containing a single copy of mutant GluN2A (L812M/2A) showed an intermediate dual exponential glutamate deactivation time course with a τ_W of 112 ms (n = 12; Fig. 3c, left; Table 2). The NMDAR current response upon briefly moving the cell into the glutamate solution to mimic the synaptic activation showed a similar tendency of the GluN2A–L812M mutation to slow the deactivation time course (Fig. 3c, right). Furthermore, the receptors containing a single copy of mutant GluN2A (L812M/2A) showed an intermediate slowing of the glycine deactivation time course that could also be described by two exponential components with τ_W of 315 ms (n = 7; Fig. 3d, left; Table 2), compared with τ_W 83 ms for 2A/2A and 784 ms for L812M/L812M. The data from experiments moving the cell briefly into the glycine solution show a similar intermediate effect of the GluN2A–L812M mutation on slowing the glycine deactivation time course (Fig. 3d, right). These data

Figure 3 | The L812M mutation prolongs deactivation time course. (a,b) The L812M mutation prolongs deactivation time course of di-heteromeric GluN2A receptors. (a) The normalized representative current response of di-heteromeric NMDARs (WT 2A and L812M) to 1 mM glutamate (left: long application (1 s) is shown; right: brief application (5 ms); 50 μM glycine in all solutions) (V_HOLD – 60 mV). L812M (black) prolongs the deactivation time course compared with the WT 2A receptors (grey). (b) The normalized representative current response of di-heteromeric NMDARs to 1 mM glycine is shown (left: long application; right: brief application; 30 μM glutamate in all solutions) (V_HOLD – 60 mV). (c,d) The L812M mutation prolongs the deactivation time course of tri-heteromeric GluN2A receptors. (c) A representative current response is shown for tri-heteromeric NMDARs with 0, 1 or 2 copies of the L812M mutation in each complex (2A/2A, L812M/2A and L812M/L812M) to 1 mM glutamate (left: long application; right: brief application; 50 μM glycine in all solutions). (d) The normalized representative current response is shown for tri-heteromeric NMDA receptors to 1 mM glycine (left: long application; right: brief application; 30 μM glutamate in all solutions) (V_HOLD – 60 mV).
Table 2 | Summary of deactivation time course.

| Glutamate deactivation | Di-heteromeric receptors | Triheteromeric receptors |
|------------------------|--------------------------|--------------------------|
|                        | WT 2A                    | 2A-L812M                 | 2A/2A | L812M/2A | L812M/L812M |
| Amplitude (peak, pA/pF) | 121 ± 19                 | 123 ± 19                 | 98 ± 26 | 90 ± 22 | 72 ± 17 |
| Amplitude (SS, pA/pF)  | 96 ± 17                  | 99 ± 13                  | 67 ± 16 | 50 ± 9.7 | 67 ± 16 |
| t½/ t½peak             | 0.83 ± 0.05              | 0.86 ± 0.05              | 0.71 ± 0.04 | 0.67 ± 0.06 | 0.92 ± 0.02 |
| Rise time (ms)         | 6.3 ± 0.5                | 7.1 ± 0.4                | 6.9 ± 0.6 | 6.8 ± 0.3 | 6.6 ± 1.1 |
| tFAST (ms)             | 37 ± 4.1                 | 264 ± 28                 | 30 ± 2.2 | 56 ± 3.9 | 201 ± 18 |
| tSLOW (ms)             | 488 ± 177               | 1,362 ± 152              | 179 ± 27 | 354 ± 27 | 1,201 ± 143 |
| %tFAST                 | 87%                     | 82%                      | 87%     | 74%     | 74%     |
| t½W (ms)               | 47 ± 4.6                | 443 ± 47*                | 40 ± 2.9 | 112 ± 8.9† | 348 ± 32† |
| Charge transfer, pA x ms/pF | 4,565                  | 44,008                  | 2,687    | 5,589    | 29,378   |
| n                      | 11                      | 10                      | 10       | 12       | 6       |

Glycine deactivation

|                        | 2A/2A | L812M/2A | L812M/L812M |
|------------------------|-------|----------|-------------|
| tFAST (ms)             | 44 ± 6.5 | 43 ± 45  | 56 ± 4.2   | 138 ± 7.9 | 413 ± 109 |
| tSLOW (ms)             | 449 ± 78 | 1,237 ± 130 | 428 ± 91 | 698 ± 96 | 1,467 ± 103 |
| %tFAST                 | 95%   | 68%      | 90%        | 68%      | 67%      |
| t½W (ms)               | 63 ± 9.6 | 691 ± 66* | 83 ± 10   | 315 ± 41† | 784 ± 112† |
| Charge transfer, pA x ms/pF | 3,906   | 60,775  | 1,851      | 6,938    | 17,725   |
| n                      | 5     | 6        | 6          | 7        | 5        |

The data were expressed as mean ± s.e.m.

Glutamate deactivation time course tau were from prolonged application (1-1.5 s). There were no significant effects of the mutation on the response rise time (9.5 ms for WT 2A; 9.3 ms for 2A-L812M; 8.0 ms for 2A/2A; 10 ms for L812M/2A; 11 ms L812M/L812M) and amplitude (82 pA for WT 2A; 88 pA for 2A-L812M; 22 pA for 2A/2A; 22 pA for L812M/2A; 23 pA for L812M/L812M) when glycine was applied with background glutamate.

Copyright © 2014 Macmillan Publishers Limited. All rights reserved.
single-channel properties from patches that contained one active channel with GluN2 subunit composition of 2A/2A, L812M/2A and L812M/L812M. Methanethiosulfonate ethylammonium (MTSEA) modification of the A652C mutation in GluN1 SYTANLAFF gating region can lock receptors open, rendering the degree of potentiation reciprocally related to the open probability prior to MTSEA treatment. Use of this approach to estimate open probability yielded the same result as data from one active channel in excised outside-out patches (Supplementary Fig. 2), confirming a dominant effect on channel gating by a single copy of the mutant receptor. These data indicate that the mutant channels open more often resulting in a profound overactivation of the NMDARs and enhanced neuronal excitability. This situation almost certainly renders neurons vulnerable to excitotoxic neuronal death.

Molecular basis for enhanced NMDA receptor function. The residue corresponding to GluN2A–Leu812 is conserved for the GluN1 and GluN2 NMDAR subunits. We hypothesized that this region may play an important role in channel gating. To test this idea, we first determined whether changes in agonist potency were associated with the L812M mutation transferred into other GluN subunits at the same position. We substituted Met for Leu in other GluN subunits at the corresponding position, leading to the generation of GluN1–L808M, GluN2B–L813M, GluN2C–L810M, GluN2D–L840M. The potency (EC50 values) of glutamate and glycine were evaluated for recombinant NMDARs expressed in Xenopus oocytes. The GluN1–L808M mutation co-expressed with GluN2A increased glutamate potency by 9.2-fold (EC 50: 0.39 versus 3.6 μM for WT 2A) and glycine potency by 9.1-fold (EC50: 0.12 versus 1.1 μM for WT) (Fig. 5a,e; Table 4). GluN1–L808M when co-expressed with GluN2B similarly increased glutamate potency by 6.8-fold (Fig. 5b; 0.16 versus 1.3 μM for WT 2B). Likewise, all GluN2 subunits harbouring the L–M mutation showed strongly enhanced potency of both glutamate and glycine (Fig. 5b–d).

**Figure 4 | The L812M mutation alters the single-channel properties.** Steady-state recordings from an outside-out patch containing a single active NMDAR with two WT leucine residues at GluN2A position 812 (2A/2A; a), a single copy of mutation L812M (L812M/2A; b) and two copies of L812M (L812M/L812M; c). Unitary currents were activated in these excised patches by 1 mM glutamate and 50 μM glycine. (d–f) The pooled open duration and shut duration histogram are shown for four single-channel recordings. Open duration histograms were made from 42,302 openings for 2A/2A, 80,303 openings for L812M/2A, 74,138 openings for L812M/L812M. Closed duration histograms were made from 42,299 events for 2A/2A, 80,301 events for L812M/2A, 74,134 events for L812M/L812M.
Based on the profound increase in charge transfer predicted for EPSCs from our data as well as the increase in activation by low levels of non-synaptic agonist, we hypothesize that these mutations cause NMDAR hyperactivity and consequently intractable seizures, abnormal development and possible excitotoxic neuronal loss.

For this patient, it seems likely that the early-onset epileptic encephalopathy could be due to the reduced block by extracellular Mg$^{2+}$, a >10-fold predicted increase in charge transfer occurring during synaptic transmission, as well as the strong increase in open probability. The greatly increased current flow through GluN2A-containing NMDARs into principle neurons should provide excessive excitatory drive, facilitating or triggering the development of clinically relevant epileptiform activity and perhaps seizures. Compounding this excessive excitatory drive will be activation of NMDARs by low levels of glutamate and/or glycine that would normally be insufficient to induce NMDAR-mediated current flow. Our results with NMDARs that contain a single copy of the mutant receptor show that overactivation does not require two copies of the mutant subunit. This enhanced excitatory drive from all receptors with at least one copy of the mutation could lead to excitotoxic neuronal death, a process long known to occur following overactivation of NMDARs$^{35,36}$. This
Neuronal loss may result in cognitive impairment, motor dysfunction and other neurological problems. Our functional data show that the GluN2A–L812M linker region mutation influences several key features of NMDARs. The proximity of this mutation to a site for allosteric regulation suggests a possible avenue for therapeutic exploration.

## Methods

### Whole-exome sequencing

DNA was obtained and sequenced based upon clinical protocol 76-HG-0238, ‘Diagnosis and Treatment of Patients with Inborn Errors of Metabolism and other Genetic Disorders’, approved by the NHGRI Institutional Review Board. The parents gave written, informed consent for medical and genetic studies designed to reach diagnoses in the affected children.

Exome sequencing was performed using genomic DNA extracted from peripheral blood. In-solution exome capture was performed according to the manufacturer’s protocol with SureSelect Human All Exon Kits (Agilent Technologies, Santa Clara, CA, USA) or the TruSeq Exome Enrichment Kit (Illumina, San Diego, CA, USA). Alignment to the human genome reference sequence (UCSC assembly hg18, NCBI build 36) was carried out using the Efficient Local Alignment of Nucleotide Data algorithm (Eland, Illumina, Inc.). Failed was used in such a way that PE reads were aligned independently, and those that aligned uniquely were grouped into genomic sequence intervals of ~100 kb. Reads that failed to align were binned with their PE mates without discarding. Crossmatch, a Smith–Waterman-based local alignment algorithm, was used to align binned reads to their respective 100 kb genomic sequence, using the parameters –minscore 21 and –masklevel 0 (http://www.phrap.org). Genotypes were called using a Bayesian genotype caller, Most Probable Genotype (MPG). To facilitate identification of de novo variants we restricted analysis of exome sequence data to regions of the genome that were routinely well covered and consistently genotyped in 147 exomes from the UDP cohort and 561 exomes from the Clinseq cohort (unaffected individuals). De novo mutations in these regions were identified using a Boolean exclusion filter that selected for variants not present in any other UDP exome and that were at positions for which >50% of the Clinseq exomes had a homopolymer genotype call. The de novo variants identified were then further filtered to exclude variants present in sequence data derived from unaffected individuals (that is, the Clinseq cohort or the 1 K genome database of April 2011), in known pseudogenes, or in known artifacts in exome sequencing. Among the variants identified by this process was the c.2434C>T (p.L812M) mutation in exon13 of GRIN2A.

### Mutagenesis

Site-directed mutagenesis was performed using the QuikChange protocol with Pfu DNA polymerase (Stratagene, La Jolla, CA, USA) to replicate the parental strand with desired mismatch incorporated into the primer. Methylation parental DNA template was digested with DpnII. The nicked double-stranded mutant DNA was transformed into TOP10 Competent Cells (Life Tech, Grand Island, NY, USA). The mutations were verified by sequencing through the region of the mutations. The cDNA for WT human NMDA subunit GluN1-1a (hereafter GluN1), GluN2A, GluN2B and GluN2D were in pC1-neo (GenBank accession codes: NP_015566, NP_000824, NP_000825 and NP_000827). The cDNAs for

---

### Table 4 | Summary of agonist EC50 values for Leu–Met mutations at the position corresponding to GluN2A–Leu812 in all other GluN1 and GluN2 subunits.

| Glutamate EC50, µM | Glycine EC50, µM |
|-------------------|----------------|
| WT 2A             | 3.6 ± 0.26 (13) | 1.1 ± 0.04 (7) |
| 2A-L812G          | 1.4 ± 0.03 (10) | 0.24 ± 0.03 (9) |
| 2A-L812T          | 0.31 ± 0.00 (12) | 0.10 ± 0.01 (10) |
| 2A-L812C          | 0.20 ± 0.01 (10) | 0.20 ± 0.02 (12) |
| 2A-L812Q          | 0.06 ± 0.01 (10) | 0.14 ± 0.01 (13) |
| 2A-L812S          | 0.4 ± 0.01 (10) | 0.10 ± 0.01 (14) |
| 2A-L812G          | 1.2 ± 0.04 (10) | 0.23 ± 0.01 (12) |
| 2B-L812M          | 0.19 ± 0.01 (6) | 0.07 ± 0.007 (10) |
| 2B-L812Q          | 0.16 ± 0.01 (10) | 0.06 ± 0.005 (11) |
| 2B-L812S          | 0.05 ± 0.006 (14)* | 0.016 ± 0.002 (13)* |

The data were expressed as mean ± s.e.m. (n).

*P < 0.05 compared with corresponding WT; unpaired t-test.

### Table 5 | Summary of agonist EC50 values for multiple substitutions at GluN2A–Leu812.

| Glutamate EC50, µM | Glycine EC50, µM |
|-------------------|----------------|
| WT 2A             | 3.6 ± 0.4 (8) | 1.1 ± 0.2 (6) |
| 2A-L812G          | 1.4 ± 0.03 (10) | 0.24 ± 0.03 (9) |
| 2A-L812T          | 0.31 ± 0.00 (12) | 0.10 ± 0.01 (10) |
| 2A-L812C          | 0.20 ± 0.01 (10) | 0.20 ± 0.02 (12) |
| 2A-L812Q          | 0.06 ± 0.01 (10) | 0.14 ± 0.01 (13) |
| 2A-L812E          | 0.90 ± 0.07 (10) | 0.16 ± 0.01 (12) |

The data were expressed as mean ± s.e.m. (n).
Zinc experiments were performed in the tricine solution at (10 mM) pH 7.3. ZnCl$_2$ solution was made fresh every day and added directly to the recording solution to obtain the desired nominal Zn$_2$ solution (10 mM) were made fresh every day and added directly to the recording solution. The constructs for tri-heteromeric receptors (GluN1/GluN2A/GluN2B) were provided by Drs S. Heinemann (Salk Institute), S. Nakanishi (Osaka Bioscience Institute), and P. Seeburg (University of Heidelberg). The constructs for tri-heteromeric receptors (GluN1/GluN2A/GluN2C) were provided by Drs S. Heinemann (Salk Institute), WT rat GluN1 (GenBank accession code: U08261), GluN2A (D13211), and GluN2C (M91563) were provided by Drs S. Heinemann (Salk Institute), and P. Seeburg (University of Heidelberg). The constructs for tri-heteromeric receptors (GluN1/GluN2A/GluN2C) were provided by Drs S. Heinemann (Salk Institute), and P. Seeburg (University of Heidelberg).

The curve with Equation 1 and Equation 2, respectively:

\[ \text{Response} = \frac{\text{Amplitude}_{\text{FAST}}}{\text{tau}_{\text{FAST}}} \left(\frac{1}{1 + \left(1 - \frac{C}{C_0}\right)^{\text{Amplitude}_{\text{SLOW}}}/\text{tau}_{\text{SLOW}}} \right) \]

where $C$ is the concentration of agonist that causes a half maximal response, and $n$ is the slope factor of the response curve.

\[ \text{Response} = (100 \% - \text{minimum}) \left(1 + \left(\frac{\text{IC}_{50}}{C}\right)^n\right) + \text{minimum} \]

where minimum is the residual percent response in saturating concentration (constrained to $>0$) of Mg$^{2+}$, protons or zinc, $IC_{50}$ is the concentration of antagonist that causes half maximal inhibition, and $n$ is the slope factor.

**Preparation and transfection of HEK 293 cells.** HEK 293 cells (ATCC, Manassas, VA, USA) or HEK 293 Tet-On Advanced cells (Clontech Laboratories, Mountain View, CA, USA) were plated on glass coverslips coated in 100 µg ml$^{-1}$ poly-l-lysine and incubated at 37 °C (5% CO$_2$ in DMEM/Glutamax) supplemented with 10% dialyzed fetal bovine serum and 10 µg ml$^{-1}$ streptomycin, G418 sulphate (150 µg ml$^{-1}$, Invivogen, San Diego, CA, USA) was added into the medium for HEK 293 Tet-On Advanced cells. HEK 293 cells were electroporated using the calcium phosphate method$^{64}$ with plasmid cDNAs encoding green fluorescent protein (GFP), GluN1 and GluN2A or GluN2A-L812M (ratio was 5:1:1) for di-heteromeric receptor experiments. HEK 293 Tet-On Advanced cells were used for tri-heteromeric receptor experiments and plasmid cDNAs encoding GFP, GluN1/GluN2A, or L812M/2A and L812M/L812M were transfected at a ratio of 1:1:1:1. In di-heteromeric receptor experiments all receptor subunit cDNA were in the pCI-neo, whereas GluN1 expression was under the control of the Tet-On inducible promoter in the pTRE-tight vector (Clontech Laboratories) in tri-heteromeric receptor experiments. The mixed plasmid DNA (0.5 µg) was co-transfected with 6.25 µg of 2.7-kb insert HCMV IE expression vector (50 mM BES, N,N-Bis(2-hydroxyethyl)taurine, 280 mM NaCl, 1.5 mM Na$_2$HPO$_4$, pH adjusted to 6.95 with HCl) was added, and the mixture was incubated for 5 min at room temperature. Calcium phosphate-DNA solution (50 µl per well) was added directly to the plate and the mixture was swirled gently and incubated for 4 h at 37 °C. After transfection, the cells were incubated overnight post-transfection in standard media supplemented with NMDAR antagonists (200 µM D,L-2-amino-5-phosphonovalerate, 100 µM 7-chlorokynurenic acid and 1.000 µM MgCl$_2$) for both HEK 293 Tet-On Advanced cells to express GluN1. The following day, ~24 h after transfection, the cells were used for patch-clamp recording (whole-cell voltage-clamp recordings and outside-out single channel recordings).

**Whole-cell and single-channel voltage-clamp recordings.** Whole-cell voltage-clamp recordings were performed on transfected HEK 293 cells at ~60 °C using an Axopatch 200b amplifier at room temperature (23 °C). Recording electrodes were made from thick-walled glass pipettes (G150-4, Warner Instruments Inc., Hamden, CT, USA) pulled using a vertical puller (Narishige P-10, Tokyo, Japan). The solution contained (in mM) 150 NaCl, 10 HEPES, 30 D-mannitol, 3 KCl, 1.0 CaCl$_2$, and 0.01 EDTA at 23 °C and pH 7.4. This level of EDTA will chelate with exceptionally high-affinity mM levels of contaminant transition metals (zinc, iron, manganese, and so on) but not significantly alter the extracellular Ca$^{2+}$ concentration. Rapid solution exchange with open tip in exchange times (<1 ms) for the solutions around the cell was achieved with a two-barreled theta-glass pipette controlled by a piezo-electric transducer (Burleigh Instruments, Newton, NJ, USA) and the data were analysed by ChanneLab (Synaptosoft, Decatur, GA, USA)$^{65}$. Deactivation time course following removal of glutamate was fitted with Equation 3:

\[ \text{Response} = \text{Amplitude}_{\text{FAST}} \left(\frac{1}{1 + \left(1 - \frac{C}{C_0}\right)^{\text{Amplitude}_{\text{SLOW}}}/\text{tau}_{\text{SLOW}}} \right) + \text{Amplitude}_{\text{SLOW}} \left(\frac{1}{1 + \left(1 - \frac{C}{C_0}\right)^{\text{Amplitude}_{\text{SLOW}}}/\text{tau}_{\text{SLOW}}} \right) \]

Single-channel recordings of outside-out patches excised from transfected HEK 293 cells were recorded at ~80 mV (digitized at 40 kHz, filtered at 8 kHz) using an Axopatch 200b amplifier at room temperature (23 °C). Recording electrodes were made from thick-walled glass pipettes (G150-4, Warner Instruments Inc., Hamden, CT, USA) pulled using a vertical puller (Narishige P-10, coated with Sylgard (Dow Corning, Midland, MI, USA) and then fire-polished to ~7–9 MΩ. The internal solution was the same as that used for the whole-cell voltage-clamp recording. The solution was bath-applied by gravity-fed perfusion system and agonists (100 µM glutamate and 100 µM CaCl$_2$) was assessed by measuring reversal potential (V$_{rev}$) of WT and mutant GluN1. Current–voltage curves were generated by applying a ramp protocol over ~90 to 30 mV (800 ms) after stepping to ~90 mV from a holding potential of ~60 mV. To minimize voltage changes, a 3 M KCl agarose (0.5%) bridge was used. Liquid junction potential between the extracellular and internal solution were measured (1.8 mM CaCl$_2$) using an Axopatch 200b amplifier at room temperature (23 °C). The relative Ca$^{2+}$ permeability was calculated as the ratio of total open duration divided by the sum of total open and closed duration.

**Evaluation of relative Ca$^{2+}$ permeability.** The relative Ca$^{2+}$ permeability (PCa/PCs) was assessed by measuring reversal potential (V$_{rev}$) of WT and mutant NMDA receptors (GluN1$^+$ or Ca$^{2+}$$^+$) in the cell-attached configuration (Ca$^{2+}$$^+$ or Ca$^{2+}$$^+$, or Ca$^{2+}$$^+$ and Mg$^{2+}$$^+$) solution contained (in mM) 140 mM NMDG, 10 mM HEPES and 1.8 mM Ca$^{2+}$, adjusted to pH 7.2 by addition of HCl. The extracellular Ca$^{2+}$ solution (143 mM) consisted of 140 mM Ca$^{2+}$ and 10 mM HEPES, adjusted to pH 7.2 by addition of CaSO$_4$. The intracellular recording solution contained (in mM) 125 CsCl, 10 HEPS, 10 EGTA (pH 7.2 by addition of CaCl$_2$), and the solution was bath-applied by gravity-fed perfusion system and agonists (100 µM glutamate and 100 µM glycine) were applied by fast-perfusion system as for the experiments of deactivation time course. The whole-cell voltage-clamp recordings were performed on HEK 293 cells co-transfected with GluN1, GFP and either WT GluN2A, GluN2A(L812M) or WT GluN2D as a positive control. Current–voltage curves were generated by applying a ramp protocol over ~90 to 30 mV (800 ms) after stepping to ~90 mV from a holding potential of ~60 mV. To minimize voltage changes, a 3 M KCl agarose (0.5%) bridge was used. Liquid junction potential between the extracellular and internal solution were measured (~12.6 mV in 1.8 mM Ca$^{2+}$ extracellular solution and used to correct for the holding potential. The current–voltage relationship was fitted by the following Equation 4:

\[ F(V) = \frac{I_{\text{max}}}{1 + e^{-c(V_{\text{rev}} - V)/V_C}} + C \]

The reversal potential was determined as the voltage at which the fitted curve crossed the abscissa. Relative Ca$^{2+}$ permeability was calculated with a modified
Lewis equation (Equation (5)) \( V_{rev}(Ca) - V_{rev}(Cs) = \frac{RT}{F} \ln \left( \frac{2 [Ca^{2+}] \times [Cs]}{[Ca]_{rev} \times F} \right) \) (5)

All reagents were purchased from Sigma. All experiments were performed on two or more separate cell preparations (injected oocytes from different frogs or separate cultures of HEK cells). Data are expressed as mean ± s.e.m. and analysed statistically using unpaired t-test and one-way ANOVA with Tukey post hoc test. Significance for all tests was set at \( P < 0.05 \). Error bars in all figures are s.e.m.

References

1. Akazawa, C., Shigemoto, R., Besho, Y., Nakanishi, S. & Mizuno, N. Differential expression of five N-methyl-D-aspartate receptor subunit mRNAs in the cerebellum of developing and adult rats. J. Comp. Neurol. 347, 150–160 (1994).
2. Monyer, H., Burnashe, N., Laurie, D. J., Sakmann, B. & Seeburg, P. H. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 12, 529–540 (1994).
3. Trayanlis, S. F. et al. Glutamate receptor ion channels: structure, regulation, and function. Pharmacology 62, 405–496 (2010).
4. Ghasemi, M. & Schachter, S. C. The NMDA receptor complex as a therapeutic target in epilepsy: a review. Epilepsy Behav. 22, 617–640 (2011).
5. Coyle, J. T., Tsai, G. & Goff, D. Converging evidence of NMDA receptor dysfunction in the pathophysiology of schizophrenia. Ann. NY Acad. Sci. 1003, 318–327 (2003).
6. Hallet, P. J. & Standaert, D. G. Rationale for and use of NMDA receptor antagonists in Parkinson’s disease. Pharmacol. Ther. 102, 155–174 (2004).
7. Arning, L. et al. NR2A and NR2B receptor gene variations modify age at onset in Huntington disease. Neurogenetics 6, 25–28 (2005).
8. Fan, M. M. & Raymond, L. A. N-methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington’s disease. Prog. Neurobiol. 81, 272–293 (2007).
9. Saft, C. et al. NMDA receptor gene variations as modifiers in Huntington disease: a replication study. PLoS Curr. 3, RRN1247 (2011).
10. Hamza, T. H. et al. Genome-wide environment study identifies glutamate receptor gene GRIN2A as a Parkinson’s disease modifier gene via interaction with coffee. PLoS Genet. 7, e1002237 (2011).
11. Tarabeux, J. et al. Rare mutations in N-methyl-D-aspartate glutamate receptors in autism spectrum disorders and schizophrenia. Transl. Psychiatry 1, e55 (2011).
12. Endele, S. et al. Mutations in GRIN2A and GRIN2B encoding regulatory subunits of NMDA receptors cause variable neurodevelopmental phenotypes. Nat. Genet. 42, 1021–1026 (2010).
13. Hamdan, F. F. et al. Excess of de novo deleterious mutations in genes associated with glutamatergic systems in nonsyndromic intellectual disability. Am. J. Hum. Genet. 88, 306–316 (2011).
14. de Ligt, J. et al. Diagnostic exome sequencing in persons with severe intellectual disability. Nat. Genet. 42, 1921–1929 (2010).
15. Lemke, J. R. et al. Mutations in GRIN2A cause idiopathic focal epilepsy with rolandic spikes. Nat. Genet. 45, 1067–1072 (2013).
16. Lesca, G. et al. GRIN2A mutations in acquired epileptic aphasia and related childhood focal epilepsies and encephalopathies with speech and language dysfunction. Nat. Genet. 45, 1061–1066 (2013).
17. Sobolevsky, A. I., Rosconi, M. P. & Gouaux, E. X-ray structure, symmetry and mechanism of an AMPA-subtype glutamate receptor. Nature 462, 745–756 (2009).
18. Heron, S. E. et al. De novo SCN1A mutations in Dravet syndrome and related epileptic encephalopathies are largely of paternal origin. J. Med. Genet. 47, 137–141 (2010).
19. Mastrangelo, M. & Leuzzi, V. Genes of early-onset epileptic encephalopathies: from genotype to phenotype. Pediatr. Neurol. 46, 24–31 (2012).
20. Kohda, K., Wang, Y. & Yuzuki, M. Mutation of a glutamate receptor motif reveals its role in gating and delta2 receptor channel properties. Nat. Neurosci. 3, 315–322 (2000).
21. Jones, K. S., VanDongen, H. M. & VanDongen, A. M. The NMDA receptor M3 segment is a conserved transduction element coupling ligand binding to channel opening. J. Neurosci. 22, 2044–2053 (2002).
22. Sobolevsky, A. I., Rooney, L. & Wollmuth, L. P. Staggering of subunits in NMDAR channel structure and gating. J. Gen. Physiol. 129, 509–525 (2007).
23. Pierson, T. M. et al. Nmda receptor subunits and channel structure. J. Biol. Chem. 280, 29708–29716 (2005).
24. Sobolevsky, A. I., Prodromou, M. L., Yelshansky, M. V. & Wollmuth, L. P. Subunit-specific contribution of pore-forming domains to NMDA receptor channel structure and gating. J. Gen. Physiol. 129, 509–525 (2007).
25. Kazi, R. et al. Mutations in GRIN2A mutations cause epilepsy–asphyxia spectrum disorders. Nat. Genet. 45, 1073–1076 (2013).
26. Pierson, T. M. et al. GRIN2A mutation and early-onset epileptic encephalopathy: personalized therapy with memantine. Ann Clin Transl Neurol. (2014) (in press).
27. Beck, C., Wollmuth, L. P., Seeburg, P. H., Sakmann, B. & Kuner, T. NMDAR channel segments forming the extracellular vestibule inferred from the accessibility of substituted cysteines. Neuron 22, 559–570 (1999).
28. Low, C. M. et al. Molecular determinants of proton-sensitive N-methyl-D-aspartate receptor gating. Mol. Pharmacol. 63, 1212–1222 (2003).
29. Taverna, F. et al. The Lurcher mutation of an alpha-amino-3-hydroxy-5-methyl-4-isonozolepropionic acid receptor subunit enhances potencies of glutamate and converts an antagonist to an agonist. J. Biol. Chem. 275, 8475–8479 (2000).
30. Sobolevsky, A. I., Beck, C. & Wollmuth, L. P. Molecular rearrangements of the extracellular vestibule in NMDAR channels during gating. Neuron 33, 75–85 (2002).
31. Blanke, M. L. & VanDongen, A. M. The NR1 M3 domain mediates allosteric coupling in the N-methyl-D-aspartate receptor. Mol. Pharmacol. 74, 454–465 (2008).
32. Villarroel, A., Regalado, M. P. & Lerma, J. Glycine-independent NMDA receptor desensitization: localization of structural determinants. Neuron 20, 329–339 (1998).
33. Ogden, K. K. & Traynelis, S. F. Contribution of the M1 transmembrane helix and pre-M1 region to positive allosteric modulation and gating of N-methyl-D-aspartate receptors. Mol. Pharmacol. 83, 1045–1056 (2013).
34. Wei, X. et al. Exome sequencing identifies GRIN2A as frequently mutated in melanoma. Nat. Genet. 43, 442–446 (2011).
35. Teer, J. K. et al. Systematic comparison of three genomic enrichment methods for massively parallel DNA sequencing. Genome Res. 20, 1420–1431 (2010).
36. Fuentes Fajardo, K. V. et al. Detecting false-positive signals in exome sequencing. H um. Mutat. 33, 609–613 (2012).
37. Hedegaard, M., Hansen, K. B., Andersen, K. T., Brauner-Osborne, H. & Traynelis, S. F. Molecular pharmacology of human NMDA receptors. Neurochem. Int. 61, 601–609 (2012).
57. Kniazeff, J. et al. Closed state of both binding domains of homodimeric mGlu receptors is required for full activity. Nat. Struct. Mol. Biol. 11, 706–713 (2004).
58. Jackson, M. R., Nilsson, T. & Peterson, P. A. Retrieval of transmembrane proteins to the endoplasmic reticulum. J. Cell Biol. 121, 317–333 (1993).
59. Jackson, M. R., Nilsson, T. & Peterson, P. A. Identification of a consensus motif for retention of transmembrane proteins in the endoplasmic reticulum. EMBO J. 9, 3153–3162 (1990).
60. Zerangue, N. et al. Analysis of endoplasmic reticulum trafficking signals by combinatorial screening in mammalian cells. Proc. Natl Acad. Sci. USA 98, 2431–2436 (2001).
61. Margeta-Mitrovic, M., Jan, Y. N. & Jan, L. Y. A trafficking checkpoint controls NMDA receptor subunit function. Nat. Neurosci. 21, 1189–1202 (2018).
62. Traynelis, S. F., Burgess, M. F., Zheng, F., Lyuboslavsky, P. & Powers, J. L. Control of voltage-independent zinc inhibition of NMDA receptors by the NR1 subunit. J. Neurosci. 18, 6163–6175 (1998).
63. Hansen, K. B. et al. Structural determinants of agonist efficacy at the glutamate binding site of N-methyl-D-aspartate receptors. Mol. Pharmacol. 84, 114–127 (2013).
64. Yuan, H., Hansen, K. B., Vance, K. M., Ogden, K. K. & Traynelis, S. F. Control of NMDA receptor function by the NR2 subunit amino-terminal domain. J. Neurosci. 29, 12045–12058 (2009).
65. Qin, F., Auerbach, A. & Sachs, F. Estimating single-channel kinetic parameters from idealized patch-clamp data containing missed events. Biophys. J. 70, 264–280 (1996).
66. Erreger, K., Dravid, S. M., Banke, T. G., Wyllie, D. J. & Traynelis, S. F. Subunit-specific gating controls rat NR1/NR2A and NR1/NR2B NMDA channel kinetics and synaptic signalling profiles. J. Physiol. 563, 345–358 (2005).
67. Siegler Retchless, B., Gao, W. & Johnson, J. W. A single GluN2 subunit residue controls NMDA receptor channel properties via intersubunit interaction. Nat. Neurosci. 15, 406–413 S401–S402 (2012).
68. Lewis, C. A. Ion-concentration dependence of the reversal potential and the single channel conductance of ion channels at the frog neuromuscular junction. J. Physiol. 286, 417–445 (1979).
69. Wollmuth, L. P., Kuner, T. & Sakmann, B. Intracellular Mg2+ interacts with structural determinants of the narrow constriction contributed by the NR1-subunit in the NMDA receptor channel. J. Physiol. 506(Pt 1): 33–52 (1998).
70. Acker, T. M. et al. Mechanism for noncompetitive inhibition by novel GluN2C/D N-methyl-D-aspartate receptor subunit-selective modulators. Mol. Pharmacol. 80, 782–795 (2011).

Acknowledgements
We are grateful to Anel Tankovic, Phuong Le and Kevin Ogden for technical assistance. We are also grateful to Eric D. Marsh and Dimitre R. Simeonov for assistance with the clinical and molecular evaluation of the patient. This work was supported by the NINDS (NS036654, S.F.T.), by the NIH Undiagnosed Diseases Program (HHSN268201300162P, H.Y.) and by the Intramural Research Program of the National Human Genome Research Institute. TMP was also supported by the Cedars-Sinai Diana and Steve Marienhoff Fashion Industries Guild Endowed Fellowship in Pediatric Neuromuscular Diseases.

Author contributions
H.Y. designed and performed the experiments, analysed the data and wrote the paper. K.B.H. designed and performed the experiments, and wrote the paper. J.Z. performed the experiments. T.M.P. performed clinical evaluation, patient phenotyping and wrote the paper. T.C.M. supervised genomic data mining. K.V.F.F. discovered mutation. C.F.B. project supervision and validated mutation. G.G. performed clinical evaluation and patient phenotyping. D.R.A. performed clinical evaluation and patient phenotyping. C.F.B. project supervision and wrote the paper. W.A.G. managed UDP and wrote the paper. S.F.T. designed the experiments, analysed the data and wrote the paper. All authors discussed the results and implications and commented on the manuscript.

Additional information
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions

How to cite this article: Yuan, H. et al. Functional analysis of a de novo GRIN2A missense mutation associated with early-onset epileptic encephalopathy. Nat. Commun. 5:3251 doi: 10.1038/ncomms4251 (2014).