Unraveling *GRIA1* neurodevelopmental disorders: Lessons learned from the p.(Ala636Thr) variant

Nicolai Kohring Tvergaard[1], Tinatin Tkemaladze[2,3], Tommy Stödberg[4,5], Malin Kvarnung[6,7], Katrina Tatton-Brown[8], Diana Baralle[9,10], Zeynep Tümer[1,11], Allan Bayat[1,12,13,14]

[1] Department of Clinical Genetics, Kennedy Center, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark.
[2] Department of Molecular and Medical Genetics, Tbilisi State Medical University, Tbilisi, Georgia.
[3]Department of Pediatrics, Givi Zhvania Pediatric Academic Clinic, Tbilisi State Medical University, Tbilisi, Georgia.
[4] Department of Women's and Children`s Health, Karolinska Institute, Stockholm, Sweden.
[5] Department of Pediatric Neurology, Karolinska University Hospital, Stockholm, Sweden.
[6]Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden
[7]Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden
[8] St George's University Hospitals NHS Foundation Trust and St George's, University of London.
[9] Wessex Clinical Genetics Service, Princess Anne Hospital, University Hospital Southampton NHS Foundation Trust, Southampton, UK.
[10] Faculty of Medicine, University of Southampton, Duthie Building, Southampton General Hospital, Southampton, UK.
[11] Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark
[12] Department of Drug Design and Pharmacology, University of Copenhagen, Denmark.
[13] Department of Epilepsy Genetics and Personalized Medicine Danish Epilepsy Centre.
[14] Department of Regional Health Research, University of Southern Denmark.

Correspondence:
Nicolai Kohring Tvergaard
Department of Clinical Genetics, Kennedy Center
Copenhagen University Hospital, Rigshospitalet,
Blegdamsvej 9, 2100, Copenhagen Ø, Denmark
Email: nicolai.kohring.tvergaard@regionh.dk

Allan Bayat, MD
Department of Clinical Genetics,
Copenhagen University Hospital, Rigshospitalet,
Blegdamsvej 9, 2100 Copenhagen Ø, Denmark.
Email: abaya@filadelfia.dk

Title: 10 words, 84 characters

Abstract: 174 words

Manuscript: 2.907 words

References: 18

Figures and tables: 1 figure, 2 tables

Supplementary files: 1 table

Abstract

Ionotropic glutamate receptors (iGluRs), specifically α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs), play a crucial role in orchestrating excitatory neurotransmission in the brain. AMPARs are intricate assemblies of subunits encoded by four paralogous genes: *GRIA1-4*. Functional studies have established that rare *GRIA* variants can alter AMPAR currents leading to a loss- or gain-of-function. Patients affected by rare heterozygous *GRIA* variants tend to have family specific variants and only few recurrent variants have been reported. We deep-phenotyped a cohort comprising eight unrelated children and adults, harboring a recurrent and well-established disease-causing *GRIA1* variant (NM_001114183.1: c.1906G>A, p.(Ala636Thr)). Recurrent symptoms included motor and/or language delay, mild-severe intellectual disability, behavioral and psychiatric comorbidities, hypotonia and epilepsy. We also report challenges in social skills, autonomy, living and work situation, and occupational levels. Furthermore, we compared their clinical manifestations in relation to those documented in patients presenting with rare heterozygous variants at analogous positions within paralogous genes. This study provides unprecedented details on the neurodevelopmental outcomes, cognitive abilities, seizure profiles, and behavioral abnormalities associated with p.(Ala636Thr) refining and broadening the clinical phenotype.

Keywords:

GRIA1, AMPAR, syndrome, epilepsy, outcome, natural history, developmental trajectory, autonomy, treatment.

1. Introduction

Ionotropic glutamate receptors (iGluRs) are pivotal in orchestrating excitatory neurotransmission in the brain. Among them, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) are integral components with a complex structure and functional significance[1]. AMPARs are composed of four subunits, designated GluA1-4 and encoded by *GRIA1-4*[2]. Most AMPARs form heterotetrametric complexes[3]. Each GluA1-4 subunit has three key domains: the N-terminal domain (NTD), the agonist-binding domain (ABD), and the transmembrane domain (TMD) which is comprised of four helices (M1-M4)[3]. Particularly, the M3 helix forms the ion channel pore and controls gating through conformational changes upon receptor activation[3] (Figure 1). This leads to the propelling of the excitatory postsynaptic current (EPSC), resulting in depolarization of the postsynaptic membrane and neuronal firing[4]. The M3 helix harbors a string of highly conserved amino acid residues across all GLUA1-4 subunits, designated the SYTANLAAF-motif, underlining its conserved critical role in regulating channel gating[4] (Figure 1C). Rare genetic variants in the *GRIA1-4* genes can disturb AMPAR physiology, leading to neurodevelopmental disorders (NDDs)[4–7].

While developmental, cognitive, behavioral, and psychiatric abnormalities, along with seizures and cerebral malformations, have frequently been associated with *GRIA*2-4[6–10], *GRIA1* variants resulting in NDD have only been reported twice[4,11]. In one study, exome screening of 8,477 NDD patients revealed GRIA1 variants in six patients[11]. In the other study, investigation of six patients revealed that the *GRIA1* variants lead to either loss- or gain-of-function of EPSC[4]. Symptoms of these latter six patients included cognitive and developmental impairment and unspecified behavioral problems[4]. Six of the 12 reported *GRIA1* patients, carried a recurrent gain-of-function (GoF) variant: NM_001114183.1: c.1906G>A, p.(Ala636Thr)[11,4]. However, the neurodevelopmental and epilepsy phenotype were only sparsely reported, and the knowledge on natural history into adolescence and adulthood was limited.

In this study, we carried out deep-phenotyping of eight unrelated individuals between ages 3-34 with the recurrent GoF variant p.(Ala636Thr) as well as compare their symptoms to those documented in patients presenting with rare heterozygous variants at analogous positions within paralogous *GRIA* genes.

2. Methods

2.1. Study cohort

We identified patients carrying the recurrent GoF variant p.(Ala636Thr) through Genematcher[12] or an international network of epilepsy and genetic departments. We also contacted the healthcare providers of previously published patients to collect updated clinical information. Clinical and genetic information was collected from the treating physician or parent by an online questionnaire provided

through REDCap. Data included, but was not limited to, seizure history, movement disorders, developmental milestones including regression/stagnation, behavioral and psychiatric issues, broad health information (i.e., dental, surgical, urogenital findings), and information on social skills (i.e., occupation, living situation),

Developmental delay (DD) was classified as mild, moderate, severe, or profound in children five years or younger. Patients older than 5 years were classified as having mild, moderate, severe, or profound intellectual disability (ID). An anti-seizure medication was considered effective if the patient achieved a >50% reduction in seizures for a period of greater than six months. The seizure and epilepsy types were classified according to the International League Against Epilepsy Classification[13,14]. Patients were classified as having a developmental and epileptic encephalopathy (DEE) if there was evidence that seizures and/or interictal EEG abnormality negatively impacted development or intellectual disability and epilepsy (ID+E) if they had developmental impairment and seizures but no evidence of an epileptic encephalopathy. Specific epilepsy syndromes were classified according to the diagnostic criteria on the ILAE new classification[15–17]. If a particular epilepsy syndrome could not be made, then each patient was classified as DEE or ID+E.

2.2. Ethics

The study was conducted in agreement with the Declaration of Helsinki and approved by the local ethics committees. Informed consent was received from legal guardians.

2.3. Genetic identification

The GoF variant p.(Ala636Thr) was detected through genome/exome sequencing either as trio or single sequencing followed by segregation analysis of the parents using Sanger sequencing and the variant is annotated using the transcript NM_001114183.1 (GRCh38/hg38).

3. Results

We identified eight unrelated patients (two females and six males) with the p.(Ala636Thr) variant. The mean age at time of reaching a genetic diagnosis was 6.2 years (range 2-12 years) while the mean age of patients is currently 13.4 years (range 3-34 years). Patients #4 and #5 were previously reported as “Patient Stockholm 5015-11D”[11] and “Patient Stockholm 2688-10D”[11]. Further, patient #7 has also been reported previously[4]. A summary of clinical features is provided in Table 1, while Supplementary Table 1 offers a complete clinical dataset.

3.1. Neurodevelopmental outcomes

The first developmental concerns was evident in all patients prior to 24 months of life; mean age of 15 months (range 4 to 24 months) (table 1). The concerns involved abnormal motor delay and/or speech delay in all patients. All patients learned to walk; average age of sitting and walking was 8 months (range 8-11 months) and 17 months (range 16-20 months), respectively. Patients #1 and #2 had neonatal hypotonia which later resolved. Patients #3-5 and #8 were reported to have a mild degree of hypotonia. Neither joint hypermobility nor increased muscle tone was reported. Out of eight patients, three had normal gross motor function while five required support for longer distances despite being able to walk shorter distances independently; amongst these five patients, four had normal gait while one had an unsteady gait. While none of the patients reported regression in hand function, fine motor skills were impacted across the board: seven could eat with utensils, five could put on clothes, but only one could button a shirt or pants, and just three could use a zipper. Additionally, three patients could use scissors, four could brush teeth, one could write, one could draw, while a single patient had no purposeful hand usage (Supplementary Table 1).

While only #1, presently at the age of three years, remains non-verbal, it is noteworthy that all other patients exhibited delayed speech acquisition (supplementary table 1). The mean age at which initial words were spoken was 28 months, with a variation spanning from 15 to 48 months. For those who progressed to speaking in sentences, the average age was 31 months, with a range of 24 to 51 months. Among those with language abilities, verbal syntax manifested in various forms: two patients displayed normal syntax accompanied by dysarthria, three spoke in short sentences with dysarthria, one communicated through short sentences without dysarthria, while another utilized simple words without dysarthria (supplementary table 1). Only a single patient (#7) had learned to communicate with sign language. Verbal regression was only reported in #8 around the age of two years; this patient is currently 34 years old and communicates using short sentences.

All patients exhibited cognitive impairment ranging between mild (n=2), moderate (n=4) and severe (n=2) (table 1). No cognitive regression was reported (supplementary table 1).

A brain magnetic resonance imaging was available in seven patients and was found to be normal in six. Patient #5 was diagnosed with an abnormal corpus callosum and also a spinal stenosis; he later underwent decompression by laminectomy (age not reported) (supplementary table 1).

3.2. Seizures, epilepsy syndromes, and seizure outcomes

An epilepsy diagnosis was reached in 3/8 patients (37.5%) with mean age of six years (range 2-10 years) at time of seizure onset (table 1). Seizure types at onset were unknown-onset bilateral tonic-clonic seizures as none of them were caught on EEG. Despite treatment initiation, they occurred either monthly or annually; they presented either during sleep (n=1) or while awake (n=1) and could

be triggered by fever/infection (n=1). One patient (#3) subsequently developed focal non-motor seizures with impaired awareness during the follow-up period. This seizure type was observed weekly and there were no reported triggers. Epilepsy syndromes were classified as ID+E rather than DEE.

Seizure freedom was achieved in 2/3 patients (66%). The antiseizure medications that reached at least 50% seizure reduction included perampanel (#3) and oxcarbazepine (#6). None of the patients underwent vagal nerve implantation or were treated with ketogenic diet.

3.2. Behavioral and psychiatric outcomes

A wide variety of behavioral problems were reported in seven patients; difficulty in making friends (n=7), autism spectrum disorder (ASD) (n=7), difficulty in understanding social situations (n=6), attention deficit hyperactive disorder (ADHD) (n=6), limited interests and repetition (n=5), sensory seeking behavior (n=3), sensory avoiding behavior (n=3), anxiety (n=3), obsessive compulsive disorder (n=2), loss of interest in things that used to interest them (n=2), sudden onset of reduced activity (n=1). Mood swings were common and included episodes of rage (n=4), high levels of frustration (n=3), occasional aggressive outbursts towards others (n=3), or self-damaging behavior (n=1). Of the six patients diagnosed with ADHD, two were based on formal neuropsychological testing while four were based on clinical assessment (supplementary table 1). For the ASD diagnoses, four were based on formal testing, while one was based on clinical estimate.

3.3. Education, work, residence and autonomy

All seven children lived with their parents, whereas the only adult patient (#8) was fully institutionalized. The adult patient participated in a workshop tailored for people with disabilities. Patients #4-8 were older than 12 years; however, none of them were reported to have a boy or girl friend. All seven children are currently enrolled in educational institutions where they received additional support as needed. This support varied, including attendance at a regular preschool with support (n=1), a preschool for children with special needs (n=1), a regular public school with support (n=2), or a specialized school for children with special needs (n=3). The cohort reported autonomous abilities such as independently reading (n=2), counting (n=2), having friends (n=1), crossing the road safely (n=1), and taking the bus (n=1). Additionally, four patients (aged 3, 5, 8, and 14 years) remained completely dependent on others (Supplementary Table 1).

3.4. Additional outcomes

Sleep disorders were reported in two patients and included nocturnal awakening (n=2) and head banging or body rocking while falling asleep (n=1) (supplementary table 1). Extra-neurological features were rare; these included reflux, constipation and vomiting in five patients, and hyperopia,

strabismus, high palate, secretory otitis media, enuresis, slight femur rotation and hypoplastic atlas causing cervical spinal stenosis reported in one patient, respectively (supplementary table 1). No autoimmune, cardio-vascular, or pulmonary findings were reported.

4. Discussion

We carried out clinical examination of eight unrelated patients with the heterozygous p.(Ala636Thr) variant in *GRIA1*. Recurrent symptoms included delayed motor and/or speech development, mild to severe cognitive impairment, muscle hypotonia and epilepsy. This study enabled us to deep-phenotype the motor skills (gait, endurance and hand function), speech (verbal syntax and pronunciation), behavioral and psychiatric comorbidities, and sleep. It also offers the first delineation of social skills, levels of autonomy, the epileptology, living and work situation and occupational levels. While this paper primarily focuses on a recurrent variant, it marks a significant milestone in GRIA-related research by establishing a novel standard for clinical data collection and evaluation. Notably, it stands as a pioneering study to thoroughly characterize not only patient symptoms but also natural history including living conditions, occupational levels, and levels of independence. This meticulous approach not only enriches our understanding of the variant but also opens new avenues for comprehensive investigation in this field.

We also compared our cohort with the six patients carrying the same variant already reported in the literature[4,11] and were able to further delineate the phenotypical spectrum as well as strengthen previously reported associations (table 1). Although borderline ID has been reported once in the literature[11], most suffer from moderate to severe ID/DD which is supported by our findings as only two of our patients suffer from mild ID. Seizures have only been reported once before[11] with onset at age 2 years, although, there were no description of the seizure semiology. We have previously reported that patients with *GRIA3* GoF variants present with seizures before their first birthday (median 1st month of life) while seizures associated with LoF *GRIA3* variants occur later than 1st year of life (median 16 months of life)[5]. We found that GRIA1-related epilepsy is a recurrent feature that occurred on 3/8 (38%) patients with average age of onset around age 6 years (range 2-10 years). This underlines that patients with GRIA1-NDDs should be monitored for seizures beyond the first years of life. Interestingly, seizures in our cohort were treatable and 2/3 patients with epilepsy are currently seizure free while #8 has annual bilateral tonic-tonic seizures but is currently not on anti-seizure medication (table 1 and supplementary table 1).

Although rare heterozygous variants in *GRIA2-4* have been recognized as causative factors in NDDs for several years[5–7], the association of *GRIA1* with a Mendelian disorder has emerged only recently[4,11]. The recurrent GoF variant p.(Ala636Thr) greatly alters the receptor sensitivity towards Glu, leading to a 25-fold reduced half-maximally effective concentration (EC_{50})[4]. The

Ala636 residue is the third alanine in the SYTANLAAF-motif of the M3 helix that undergoes conformational changes during channel gating (Figure 1C)[4]. Specifically, in-silico models show that Ala636 exhibits nearly identical conformation across all four subunits when the gates are in their closed state[4]. This consistency is maintained by strong hydrophobic interactions with the side chains of the adjacent subunit, revealing the substitution of alanine with threonine as a destabilizing factor in closed-gate configurations, most likely due to additional bulk and polarity[4]. Given the high conservation of the SYTANLAAF-motif across all subunits, it is alluring to explore whether any pathological variation at this position, along with their associated phenotypes, have been reported in the paralogs (*GRIA2-4*). In *GRIA2-4*, the third alanine residue in the SYTANLAAF-motif is Ala643, Ala654 and Ala644, respectively[5–7]. Indeed, pathological GoF variants at these positions have been established in *GRIA2* ((p.(Ala643Val))[6], *GRIA3* ((p.(Ala654Val), p.(Ala654Pro), p.(Ala654Thr))[5], and GRIA4 (p.(Ala644Val)[18](Figure 1C). In *GRIA2-4*, the GoF variants increase glutamate potency (decreased EC_{50}), much like the recurrent variant p.(Ala636Thr) in GRIA1[5,6,18]. Table 2 compares the phenotypes reported in NDD patients with these analogous variants. While patients with the GoF *GRIA1–4* variants share common phenotypic characteristics such as ID/DD, abnormal body tone, epilepsy, and behavioral issues, there are evident distinctions. Notably, *GRIA3* variants manifest a particularly severe phenotype characterized by neonatal-onset, treatment-resistance seizures, heightened risk of early mortality, and limited or absent verbal and/or motor skills (table 2). Muscle hypertonia and hyperekplexia have so far exclusively been linked to the three *GRIA3* variants[5], whereas patients with the paralogous *GRIA* variants typically display reduced body tone (table 2).

Knowing whether a variant leads to gain- or loss-of-function may enable precision therapy, as the anti-seizure medication, perampanel, is a specific negative modulator of AMPAR[6]. Indeed, perampanel has previously been administered to a patient with the analogous variant p.(Ala643Val) in *GRIA2*[6]. A combination of ketogenic diet and perampanel successfully suppressed seizure activity in the patient and also lead to improved body tone and milestones[6]. Further, perampanel is currently prescribed to our patient #3 who has been seizure free for 6 months since treatment initiation. Although encouraging, further studies involving more patients are necessary to elucidate the genuine effect of such treatment endeavors. Since conducting large randomized controlled studies is not feasible for rare genetic disorders, N-of-1 trials have been proposed as a valid alternative for such cohorts[18]. These trials involve systematically alternating between periods of treatment and no treatment in single or small number of patients. By doing so, they allow for the comparison of treatment efficacy within the same individual, thereby minimizing the effects of individual variation and providing personalized evidence for treatment effectiveness. Therefore, future N-of-1 trials are crucial for defining the efficacy of perampanel treatment of patients with GoF *GRIA1-4* variants, including the recurrent variant p.(Ala636Thr).

5. Conclusion

In conclusion, this study investigates clinical symptoms of *GRIA1*-related neurodevelopmental disorders, specifically focusing on patients carrying the recurrent GoF variant p.(Ala636Thr). Our cohort is the largest retrospective study of patients carrying this variant, expanding the understanding of the clinical spectrum and the natural history into adulthood. Key findings include a spectrum of neurodevelopmental challenges such as cognitive and/or developmental delay, absent or delayed speech, diverse behavioral and psychiatric comorbidities, epilepsy, and a significant reliance on external support. Our study not only broadens the clinical phenotype associated with *GRIA1*-related NDDs, but also underlines the potential therapeutic interventions for patients carrying the recurrent variant p.(Ala636Thr).

Conflict of interest

None of the other authors have any conflict of interest to disclose.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Acknowledgements

We would like to thank the families for participating in this study. We would like to thank all co-authors for their contribution with clinical data.

Data availability

Anonymized data not published in this article will be made available by request from any qualified investigator.

Legends:

Figure 1: The AMPA receptor. **A)** An AMPA receptor with two Glua1 subunits (white) and two Glua2 subunits (grey), constituting a heterotetrametric complex containing an N-terminal domain (NTD), agonist binding domain (ABD), and a transmembrane domain (TBD), with four helices (M1-M4). **B)** Part of the M3 helix C-terminal amino acid sequence in *GRIA1-4* is visualized, with the SYTANLAAF motif boxed in red. All reported gain of function (GoF) variants found at the position equivalent to the recurrent variant p.(Ala636Thr) within the paralogous have been indicated.

Table 1: Clinical features of eight patients in our cohort with the recurrent p.(Ala636Thr) *GRIA1* variant.

Table 2: Comparative analysis of symptoms between patients in this cohort harboring the recurrent heterozygous p.(Ala636Thr) *GRIA1* variant and published patients manifesting rare heterozygous variants at analogous positions within paralogous *GRIA* genes.

Supplementary table 1: Detailed clinical overview of eight patients in our cohort with the heterozygous *GRIA1* variant p.(Ala636Thr).

Graphical Abstract Image: Lessons learned from patients with *GRIA1* encephalopathy.

References

1. Karakas, E., Regan, M. C. & Furukawa, H. Emerging structural insights into the function of ionotropic glutamate receptors. *Trends Biochem Sci* **40**, 328 (2015).

2. van der Spek, S. J. F. *et al.* Expression and Interaction Proteomics of GluA1- and GluA3-Subunit-Containing AMPARs Reveal Distinct Protein Composition. *Cells* **11**, 3648 (2022).

3. Kamalova, A. & Nakagawa, T. AMPA receptor structure and auxiliary subunits. *Journal of Physiology* **599**, 453–469 (2021).

4. Ismail, V. *et al.* Identification and functional evaluation of GRIA1 missense and truncation variants in individuals with ID: An emerging neurodevelopmental syndrome. *Am J Hum Genet* **109**, 1217 (2022).

5. Rinaldi, B. *et al.* Gain-of-function and loss-of-function variants in GRIA3 lead to distinct neurodevelopmental phenotypes. *Brain* **57**, 70 (2023).

6. Coombs, I. D. *et al.* A gain-of-function GRIA2 variant associated with neurodevelopmental delay and seizures: Functional characterization and targeted treatment. *Epilepsia* **63**, e156–e163 (2022).

7. Martin, S. *et al.* De Novo Variants in GRIA4 Lead to Intellectual Disability with or without Seizures and Gait Abnormalities. *AJHG* 1013–1020 (2017) doi:10.1016/j.ajhg.2017.11.004.

8. Salpietro, V. AMPA receptor GluA2 subunit defects are a cause of neurodevelopmental disorders. doi:10.1038/s41467-019-10910-w.

9. Sun, J. H. *et al.* X-linked neonatal-onset epileptic encephalopathy associated with a gain-of-function variant p.R660T in GRIA3. *PLoS Genet* **17**, (2021).

10. Rinaldi, B. *et al.* Myoclonic status epilepticus and cerebellar hypoplasia associated with a novel variant in the GRIA3 gene. **1**, 3.

11. Geisheker, M. R. *et al.* Hotspots of missense mutation identify novel neurodevelopmental disorder genes and functional domains. *Nat Neurosci* **20**, 1043 (2017).

12. Sobreira, N., Schiettecatte, F., Valle, D. & Hamosh, A. GeneMatcher: A Matching Tool for Connecting Investigators with an Interest in the Same Gene. *Hum Mutat* **36**, 928 (2015).

13. Fisher, R. S. *et al.* ILAE Official Report: A practical clinical definition of epilepsy. *Epilepsia* **55**, 475–482 (2014).

14. Fisher, R. S. *et al.* Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. *Epilepsia* **58**, 522–530 (2017).

15. Specchio, N. *et al.* International League Against Epilepsy classification and definition of epilepsy syndromes with onset in childhood: Position paper by the ILAE Task Force on Nosology and Definitions. *Epilepsia* **63**, 1398–1442 (2022).

16. Hirsch, E. *et al.* ILAE definition of the Idiopathic Generalized Epilepsy Syndromes: Position statement by the ILAE Task Force on Nosology and Definitions. *Epilepsia* **63**, 1475–1499 (2022).

17. Zuberi, S. M. *et al.* ILAE classification and definition of epilepsy syndromes with onset in neonates and infants: Position statement by the ILAE Task Force on Nosology and Definitions. *Epilepsia* **63**, 1349–1397 (2022).

18. XiangWei, W. *et al.* Clinical and functional consequences of GRIA variants in patients with neurological diseases. *Cellular and Molecular Life Sciences* **80**, 1–19 (2023).