Novel In-Frame Deletion in *GRIA3*: A Case Report

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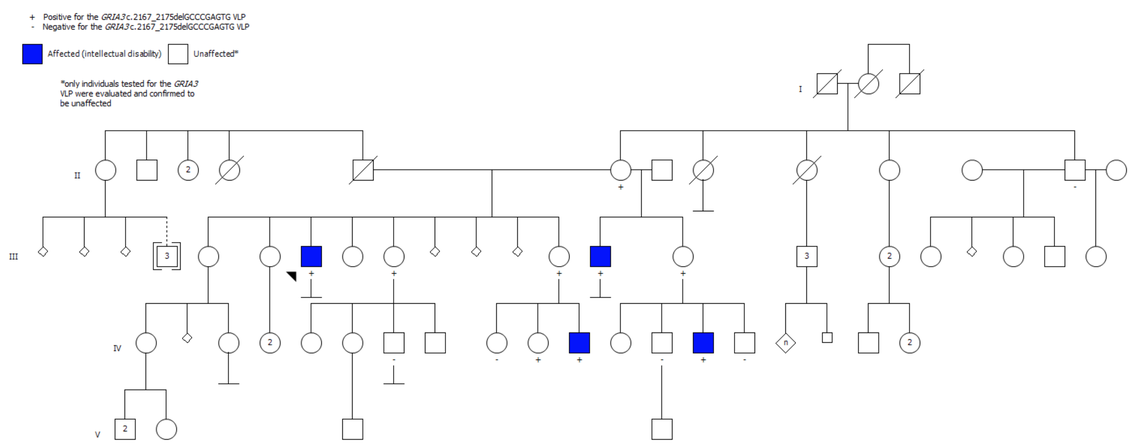
BACKGROUND

- X-linked intellectual disability (XLID) is a collection of genetically heterogeneous disorders thought to explain approximately 16% of all intellectual disability in males.
- One gene, *GRIA3*, located on chromosome Xq25, encodes glutamate receptor 3 and is known to be associated with XLID.
- Several case reports have shown mutations in *GRIA3* to be associated with varying degrees of intellectual disability, behavioral problems, autistic features, seizures, poor muscle bulk, short stature, and dysmorphic features, though available clinical and functional data is limited.
- We present a case referred for genetic testing involving a 45-year-old male proband with moderate intellectual disability, mild dysmorphic features (hypotelorism, prominent eyebrows, narrow face, absent earlobes), a speech disorder, a thyroid disorder, elevated cholesterol, and a behavior disorder.

PEDIGREE



METHODS

- A blood sample was collected from the proband and sent to Ambry Genetics (Aliso Viejo, CA) for the Ambry XLMR Next Gen Sequencing Panel™ (currently referred to as the Ambry XLID Next Gen Sequencing Panel™).
- The Ambry XLID (XLMR) Next Gen Sequencing Panel™ is a comprehensive gene sequencing assay for 81 genes associated with X-linked intellectual disability (nonsyndromic and syndromic).
- Genomic deoxyribonucleic acid (gDNA) was isolated from the patient's specimen using a standardized kit and quantified.
- Enrichment was carried out by incorporating the gDNA into microdroplets along with primer pairs designed to the target XLID gene coding exons followed by polymerase chain reaction (PCR) and Next-Generation sequencing.

RESULTS

- A novel in-frame deletion of nine nucleotides between positions 2167 and 2175 was identified in the proband: **c.2167_2175delGCCCGAGTG** (also known as p.V723del3).
- This in-frame deletion was initially considered a variant of unknown significance (VUS), as the reading frame was not impacted and there was no literature on this alteration.
- After 12 additional family members' samples were tested (see pedigree), segregation was proven to be strong in this family with a likelihood ratio of 114:1 and a LOD score of 2.055, allowing the deletion to be re-classified from a VUS to a variant, likely pathogenic (VLP) in accordance with Ambry's 5-tier variant classification scheme.
- Out of a total of 13 family members who were tested, all (4) affected males were positive, all (3) unaffected males were negative, and there were no affected females (Table 1).
- All affected males were similarly affected with moderate intellectual disability and behavioral problems, but without major health problems or congenital defects.

TABLE 1 – FAMILY MEMBERS RESULTS

	Positive	Negative	Total
Affected Male	4	0	**4**
Unaffected Male	0	3	**3**
Affected Female	0	0	**0**
Unaffected Female	5	1	**6**
Total	**9**	**4**	**13**

VARIANT INTERPRETATION

- This alteration leads to a deletion of three amino acids [alanine (A), arginine (R), and valine(V)] between codons 723 and 725, but does not cause a reading frame shift.
- Based on protein sequence alignment, these three amino acid positions are completely conserved through reptiles (Fig. 2).
- This variant is located in the PBPb functional domain of coding exon 13 in the *GRIA3* gene (Fig. 3).
- This variant was not reported in population-based cohorts in the following databases: Database of Single Nucleotide Polymorphisms (dbSNP), NHLBI Exome Sequencing Project (ESP), and 1000 Genomes Project.

FIGURE 1 – CHROMATOGRAM OF THE VARIANT

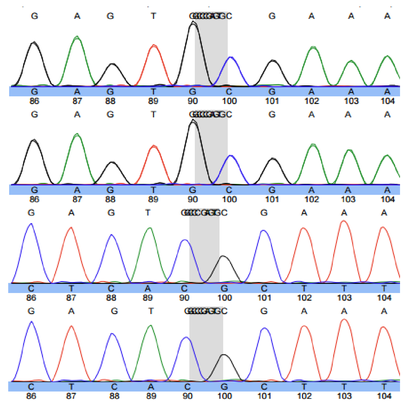
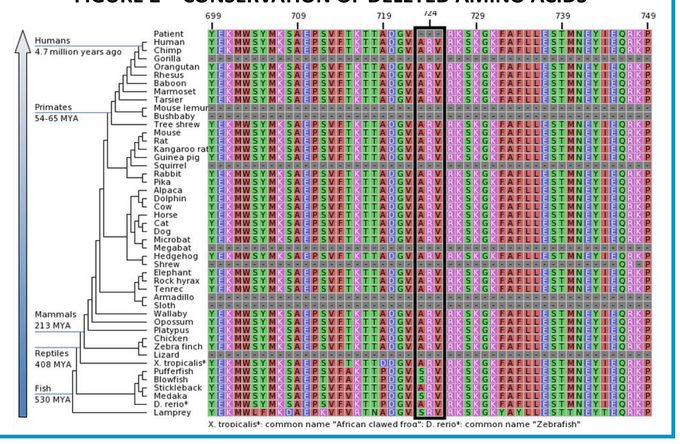


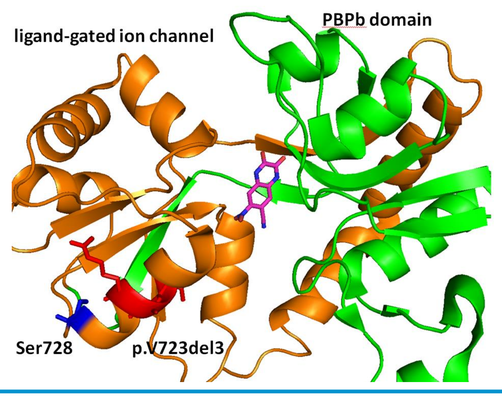
FIGURE 2 – CONSERVATION OF DELETED AMINO ACIDS



STRUCTURAL ANALYSIS

- Removal of the three residues disrupts the surface of the cavity that binds glutamate and other agonists[4] and the S728 structure which is involved in desensitization of Purkinje cells[5].
- Predicted by PROVEAN *in silico* predictor to be extremely deleterious (-19 where a value below -2 is deleterious)[6].

FIGURE 3 – THE VARIANT DISRUPTS GLUTAMATE BINDING



DISCUSSION

- Previous testing in the proband and his family members detected two translocations [t(4q;10p and t(6;9)] that do not segregate with disease in the family.
- While the affected males in this family do have significant phenotypic overlap with other individuals reported in the literature with *GRIA3* alterations (see below), the proband also has unique features which have not yet been reported in individuals with *GRIA3* alterations (absent earlobes, prominent eyebrows, thyroid disorder, and elevated cholesterol).
  - **(Philips, et al. 2014):** Detected the p.G630R (c.1888G>C) *GRIA3* alteration which segregated strongly in one large family. The majority of affected males with this alteration displayed severe intellectual disability, autistic features, behavior troubles, self injury, aggressive outbursts, dysmorphic features, brachycephaly, deep set eyes, prominent supraorbital ridges, short stature, epilepsy, and malposition of feet.
  - **(Wu, et al. 2007):** Detected four unique *GRIA3* alterations as well as a genomic deletion (0.4Mb) involving the entire *GRIA3* gene in individuals with mild to moderate intellectual disability.
  - **(Hu, et al. 2015):** Detected two unique *GRIA3* alterations in individuals with dysmorphic features and intellectual disability.
- Ambry's family study program allowed family members from this family to be tested free of charge, while still contributing to segregation analysis and an ultimate variant reclassification.

TAKE-HOME POINTS

- XLID (XLMR) Next Gen Sequencing Panel™ identified a novel causative VLP in all affected males who were tested in this family.
- The data presented here contributes additional phenotypic information regarding *GRIA3* alterations to the currently limited literature.
- Segregation analysis can be an useful tool for clinical laboratories' variant classification efforts.
- We encourage clinicians and genetic counselors to submit informative family members for diagnostic testing and/or family studies testing when available.
- We encourage continued reporting of other individuals with *GRIA3* alterations to further delineate the associated clinical phenotypes.

REFERENCES

1. Philips, et al. *Orphanet J Rare Dis.* 2014 ;11;9:49
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