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The molecular neuropathology of spinocerebellar ataxia type 23

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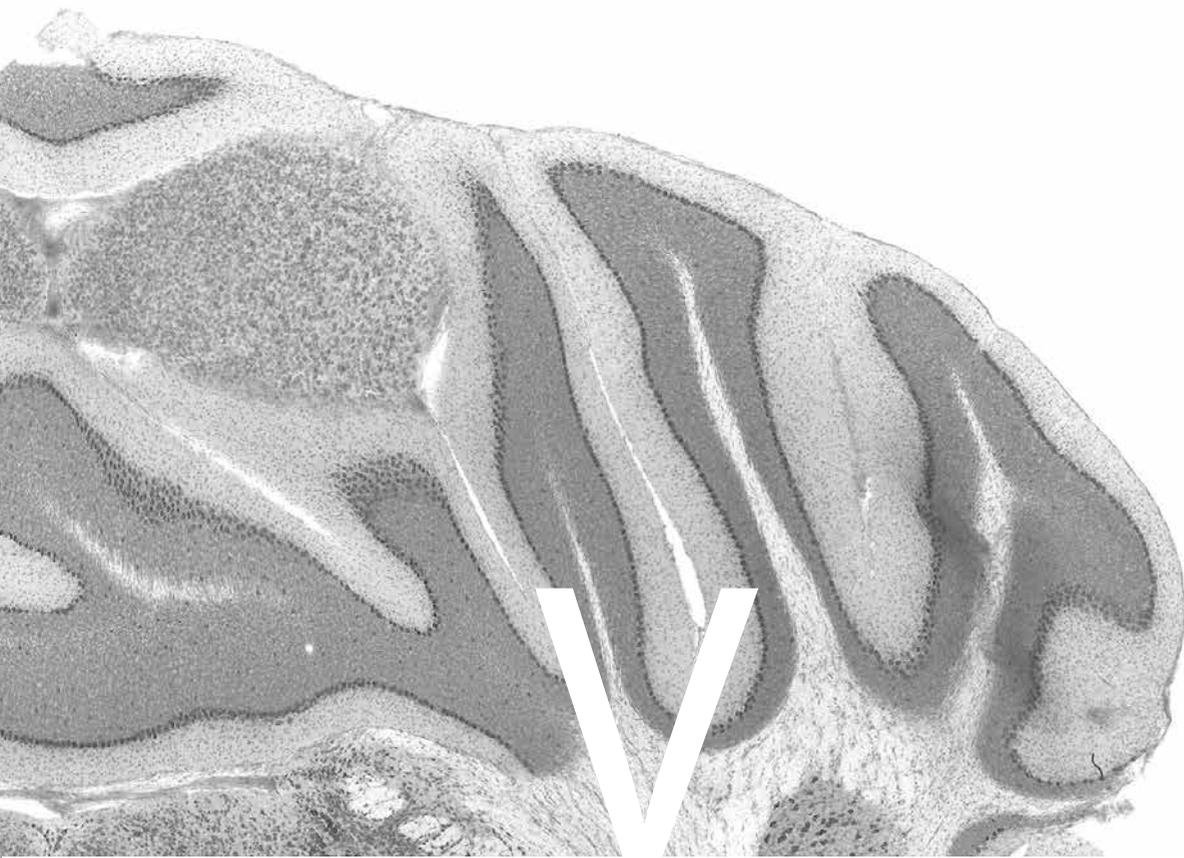
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Screening for mutations in genes encoding glutamatergic components in suspected cerebellar ataxia cases reveals a link with intellectual disability

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To the editor,

Spinocerebellar ataxias (SCA) are a large group of complex heterogeneous neurodegenerative disorders that cause cerebellar atrophy leading to motor dysfunction and ataxia¹. Despite the fact that 8 disease loci and 31 genes have been identified as causing SCA^{1,2}, approximately 30% of cases remain genetically undiagnosed. The functions of the SCA genes are diverse but they seemingly operate in shared biological pathways including synaptic transmission^{3,4}.

Glutamate receptors and transporters control proper neurotransmission and alterations of their function have been associated with diverse neurological diseases including schizophrenia, intellectual disability, epilepsy, and autism⁵. Deletions and missense mutations in the ionotropic glutamate receptor GluR2 (*GRID2*) have also been reported to cause congenital cerebellar ataxia and adult-onset spinocerebellar ataxia^{6,7}, and an autosomal-recessive congenital cerebellar ataxia was found to be caused by mutations in the metabotropic glutamate receptor 1⁸. Further, mutations in the glutamate transporter *EAAT1* cause episodic ataxia, hemiplegia and ataxia⁹.

To determine whether alterations in glutamate signalling might be a common theme in cerebellar ataxia, we screened a randomly selected cohort of 96 patients, who were all referred for SCA DNA diagnostics and thus suspected to suffer from familial or sporadic cerebellar ataxia. These cases were mostly of Dutch origin, and mutations in SCA1-3, 6, 7, 17, 19 and 23 genes were excluded. In an attempt to identify the genetic deficit we screened these cases for mutations in 39 genes involved in glutamatergic signalling (Suppl. Table 1). The complete coding regions, including exon-intron boundaries, of all genes were screened using a targeted resequencing array (Agilent technologies, USA) and sequencing was performed on a MiSeq sequencer (Illumina Inc, USA). Data analysis was performed as described previously¹⁰ and variant annotation was performed using the Cartagenia Bench Lab software (Cartagenia Inc, USA). All variants that were reported less than 0.01% in the ExAC browser (Exome Aggregation Consortium (ExAC), Cambridge, MA (<http://exac.broadinstitute.org>) [September 2015, accessed]) were excluded from further analysis. The study has been approved by the local ethics committee. Here, we report the identification of one rare and four novel variants in glutamatergic genes linked to intellectual disability and/or non-progressive gait and limb ataxia, or trunk ataxia.

We identified a novel truncating variation, c.2415C>A, p.C805*, in glutamate receptor GluA3 (*GRIA3*) in a male patient who, to our surprise, did not exhibit clear cerebellar ataxia but suffered from intellectual disability at two and a half years of age. No other family members were reported to suffer from cerebellar ataxia or intellectual disability. Genetic alterations including missense mutations in *GRIA3* have previously been reported to cause X-linked intellectual disability in humans¹¹⁻¹³, and we therefore consider this p.C805* variant pathogenic.

We identified another not-yet-reported missense variation, c.1466A>G, p.N489S, in glutaminase 2 (*GLS2*) in a young male (12 years of age) suffering from non-progressive gait and limb ataxia, dysarthria and intellectual disability starting from 1 year of age. MRI analysis showed very mild

vermis hypoplasia. Unfortunately, the brother of the patient, who displayed similar symptoms, was not available for genetic testing, and due to the unavailability of *GLS2* cDNA, the effect of the p.N489S variant on *GLS2* functioning and cellular localization could not be determined. Therefore, this variant could still be a rare benign polymorphism.

We discovered a missense variation, c.3007C>T, p.R1003W (Minor Allele Frequency (MAF) = 0.0001077; reported once in the ExAC browser), in the C-terminal cytoplasmic tail of the *N*-methyl-D-aspartate receptor subunit GluN3B (*GRIN3B*) in a female who as a child was diagnosed with coordination problems, nystagmus, dystonic features, and intellectual disability, but no clear cerebellar ataxia. The p.R1003W variant, corresponding to p.R975W in rat *Grin3b*, did not affect

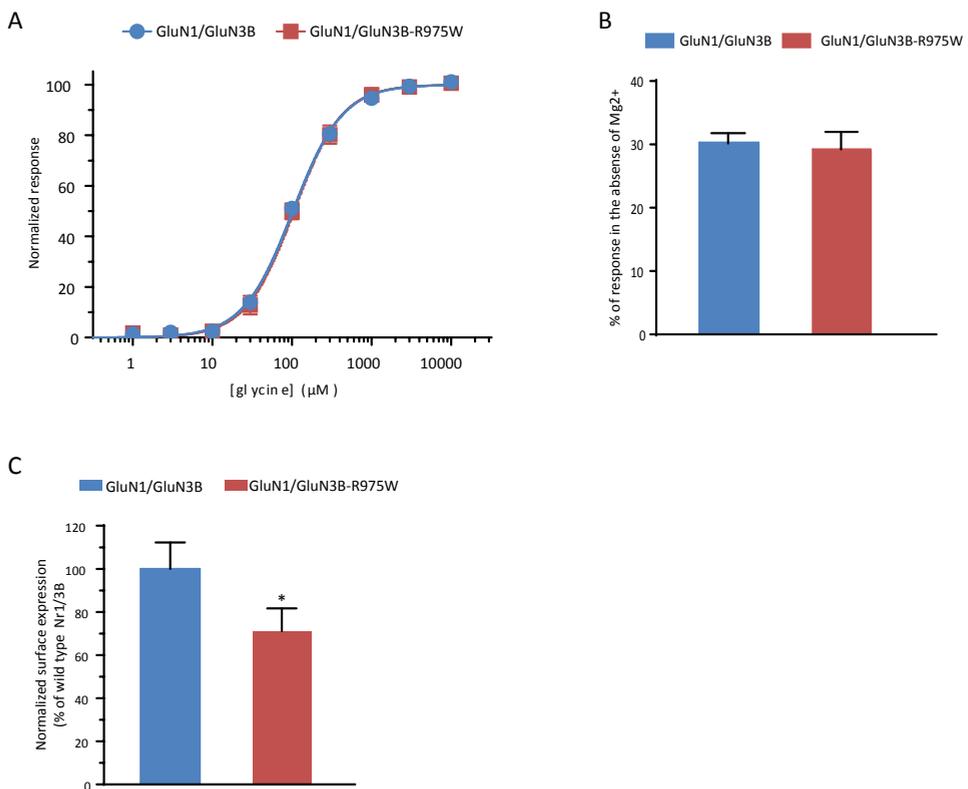


Figure 1. *GRIN3B*-Arg1003Trp displays reduced surface expression

(A) Glycine response curve of rat GluN1/GluN3B and GluN1/GluN3B-Arg975Trp receptor channels as determined by two-electrode voltage-clamp recordings in *Xenopus* oocytes. The GluN1 subunit contained Phe484Ala and Thr518Lys mutations to prevent desensitization by eliminating glycine binding to GluN1 as previously described¹⁶. GluN3B- Arg975Trp on the rat subunit corresponds to the human GluN3B- Arg1003Trp mutation. The GluN1/GluN3B- Arg975Trp channel did not show any changes as compared to GluN1/GluN3B channels. (B) The response to in the presence of 1 mM Mg²⁺ is shown as the percentage of the response in absence of Mg²⁺ (cells voltage-clamped at -80 mV). The *Grin3b*- Arg975Trp variation did not alter the Mg²⁺ block of GluN1/GluN3B- Arg975Trp receptor channels compared to GluN1/GluN3B channels. (C) Surface expression of GluN1/GluN3B and GluN1/GluN3B- Arg975Trp receptor channels in transfected HEK cells. The *Grin3b*- Arg975Trp variation reduced surface expression by approximately 30% compared to wild type GluN1/GluN3B. * $p < 0.05$

the glycine potency or Mg^{2+} block of recombinant GluN1/GluN3B receptors expressed in *Xenopus* oocytes (Fig. 1A and B). However, GluN3B-R975W did show reduced cell surface expression compared to wild type GluN3B in transfected HEK cells (Fig. 1C). Therefore, we speculate that reduced trafficking of the receptor complex may underlie the disease phenotype, however, this needs to be further investigated. Notably, null alleles of *GRIN3B* have been reported¹⁴, but were found not to be associated with motor neuron disease, suggesting that dominant negative mutations rather than haploinsufficiency of *GRIN3B* may lead to disease.

Finally, we identified a novel heterozygous frameshift, c.2523delA, p.E841fs29X, in the ionotropic glutamate receptor, GluK1 (*GRIK1*), in a male who also carried the rare c.12232T>A, p.L411* *GRIK1* allele (MAF= 0.00003314, ExAC Browser). DNA analysis was requested at the age of 62 because of trunk ataxia with dizziness, and low reflexes at a later age. No additional family members were available for testing. We showed that the p.E841fs29X variant led to a shorter GluK1 protein compared to GluK1-WT in HEK cells, whereas the rare p.L411* variation caused complete loss of GluK1 (Fig. 2A), very likely due to nonsense-mediated mRNA decay. Additionally, extracts of cells expressing GluK1-E841fs showed increased high molecular weight species compared to cells

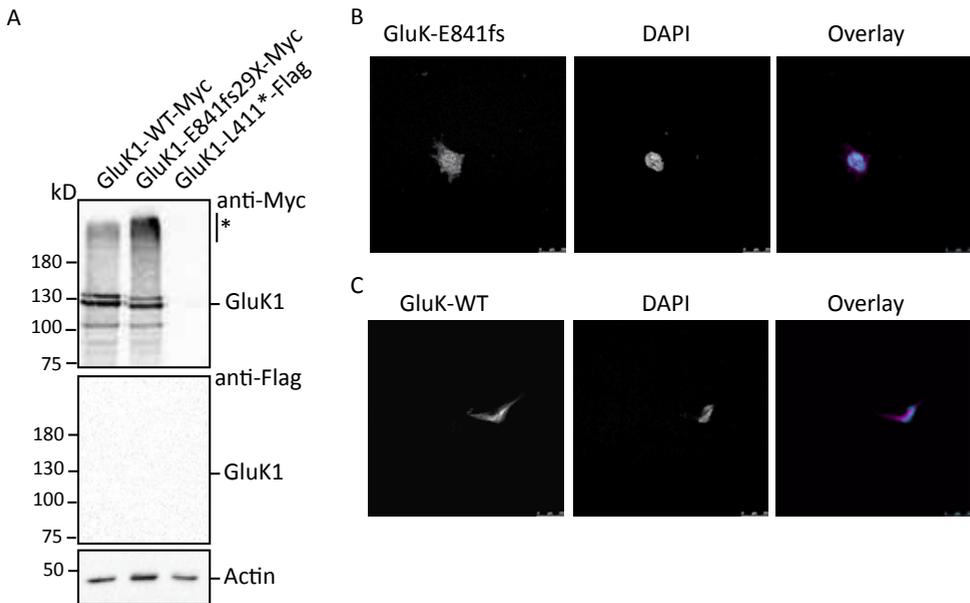


Figure 2. *GRIK1* variants affect protein expression

(A) Representative Western blot of HEK cells transfected with *GluK1*-WT-Myc, *GluK1*-Glu841fs29X-Myc, or *GluK1*-Lys411*-Flag. *GluK1*-Glu841fs29X settled lower in the gel than *GluK1*-WT, indicating that this variant leads to a shorter *GluK1* protein. *GluK1*-Lys411* protein was not detected on blots, indicating that this variant does not result in protein expression. (B-C) Representative micrographs of *GluK1* receptor complexes in non-permeabilized SH-SY5Y cells expressing *GluK1*-WT-Myc or *GluK1*-Glu841fs-Myc. *GluK1*-Glu841fs receptor channels demonstrated surface expression, however, it was reduced as compared to *GluK1*-WT (A). *GluK1*-WT displayed robust surface expression (B). Scale bars, 25 μ m.

expressing GluK1-WT, indicative of altered triton solubility of the GluK1-E841fs receptor complexes (Fig. 2A). Notably, GluK1-E841fs resulted in surface expression of the receptor (Fig. 2B), albeit lower than GluK1-WT surface expression in SH-SY5Y cells (Fig. 2C). GluK1-L411* did not display surface expression (data not shown). Whether the p.E841fs29X variation also affects receptor functioning is yet undetermined.

We hypothesize that loss of one *GRIK1* allele caused by the p.L411* variation is benign when the wild-type *GRIK1* allele is properly expressed. However, the combination of the p.L411* and p.E841fs29X variations may lead to critically altered GluK1 complexes, inducing trunk ataxia.

In summary, we have identified the first mutations in *GRIN3B* and *GRIK1* that link to intellectual disability and/or an ataxic phenotype. Additionally, to our knowledge, no human diseases are yet linked to *GLS2*. As the p.N489S variant was not previously reported, and the established role of *GLS2* in neuronal differentiation¹⁵, *GLS2* is a very promising candidate gene for a neurodevelopmental disorder such as intellectual disability.

Our findings indicate that novel variations in genes coding for glutamatergic components link more strongly to intellectual disability than they do to cerebellar ataxia. Additionally, excluding the case carrying the novel *GRIA3* variant, our findings may suggest that intellectual disability and cerebellar ataxia are biologically more closely related than previously thought. Moreover, we show that regular SCA diagnostics are sometimes requested to exclude the diagnosis cerebellar ataxia rather than confirm it, and these patients could have benefited from a more restricted clinical classification. However, with the implementation of the disease-focused gene-panels, this problem will become less prominent in the future, as closely related disorders will be tested for simultaneously. Nevertheless, when ataxia or discoordination coincides with intellectual disability, neurologists and clinical geneticists should consider screening a gene panel for intellectual disability that contains genes encoding glutamatergic components, in addition to a gene panel for movement disorders.

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