The genetics of spinocerebellar ataxia and dystonia

Nibbeling, Esther

Genetic screening of glutamatergic components in cases suspected to suffer from cerebellar ataxia reveals a link with intellectual disability

C.J.L.M. Smeets[1,*], E.A.R. Nibbeling[1,*], M.R. Fokkens[1], D. Brandenburg-Weening[1], F. Yi[2], K. N. Dorsett[2], G.C. Bullard[2], G. van der Vries[1], C.C. Verschuuren-Bemelmans[1], K.B. Hansen[2], R.J. Sinke[1], and D.S. Verbeek[1]

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1. *Department of Genetics, University Medical Centre Groningen, University of Groningen, Groningen, the Netherlands*
2. *Department of Biomedical & Pharmaceutical Sciences, University of Montana, Missoula, Montana, United States of America*

[*] *These authors contributed equally*

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To the editor,

Spinocerebellar ataxia (SCA) represents a group of complex heterogeneous neurodegenerative disorders that cause cerebellar atrophy leading to ataxia.[1] Despite the fact that more than 40 genes have been identified as causing SCA,[2] approximately 30% of cases remain genetically undiagnosed. The different SCA genes have diverse functions, but operate in shared biological pathways, including synaptic transmission.[3,4]

Glutamate signaling controls proper neurotransmission and alterations have been associated with diverse neurological diseases including intellectual disability, and autism.[5] Mutations in the ionotropic glutamate receptor GluD2 (*GRID2*) and metabotropic glutamate receptor 1 have been reported to cause congenital cerebellar ataxia and adult-onset spinocerebellar ataxia,[6,7] and autosomal-recessive congenital cerebellar ataxia [8], respectively. Additionally, mutations in the glutamate transporter *EAAT1* cause episodic ataxia, hemiplegia and ataxia.[9]

To determine whether alterations in glutamate signaling are a common theme underlying cerebellar ataxia, we screened a randomly selected cohort of 96 Dutch patients suspected to suffer from cerebellar ataxia but without mutations in the SCA1-3, 6, 7, 17, 19 and 23 genes, for mutations in 39 genes involved in glutamatergic signaling using targeted re-sequencing (for details see Supplementary Table 1). This study has been approved by the local ethics committee.

We identified a novel truncating variation, c.2415C>A, p.Cys805*, in glutamate receptor GluA3 (*GRIA3*) in a male patient, whom from two and a half years on, suffered from intellectual and motor disabilities. To date, only four truncating variations in *GRIA3* are reported in the ExAC browser, of which three are very rare (MAF <0.00001), suggesting that truncating variations in *GRIA3* are not well tolerated. Mutations in *GRIA3* have previously been reported to cause X-linked intellectual disability in humans.[5] Therefore, we consider this p.Cys805* variant pathogenic and the boy should be diagnosed with X-linked intellectual disability.

Next, we identified a novel truncating variant, c.2523delA, p.Glu841fs29*, in the ionotropic glutamate receptor, GluK1 (*GRIK1*), in a male who also carried a rare c.12232T>A, p.Lys411* *GRIK1* variant (MAF= 0.00003314, ExAC Browser). DNA analysis was requested at the age of 62 due to trunk ataxia with dizziness, and low reflexes at a later age. To investigate the pathogenicity of both variants, we transiently transfected HEK293T cells with either wild type (WT) GluK1, GluK1-Glu841fs, or GluK1-Lys411*. Immunoblotting confirmed the presence of a shorter GluK1-Glu841fs protein compared to GluK1-WT, while no GluK1 protein was observed for GluK1-Lys411* (Supplementary Figure 1A). We hypothesize that GluK1-Lys411* is very likely unstable and gets degraded. Additionally, extracts of cells expressing GluK1-Glu841fs showed increased high molecular weight

species, indicative of altered solubility of the GluK1-Glu841fs receptor complexes (Supplementary Figure 1A). To investigate whether these mutant receptors can reach the cell surface, we performed immunocytochemistry on non-permeabilized SH-SY5Y cells expressing either GluK1-WT, GluK1-Glu841fs, or GluK1-Lys411*. GluK1-Glu841fs reached the cell surface (Supplementary Figure 1B), albeit less so than GluK1-WT (Supplementary Figure 1C). Whereas GluK1-Lys411* did not display surface expression (data not shown). We hypothesize that loss of one *GRIK1* allele is benign, however, when both alleles generate modified or defective products, this leads to critically altered GluK1 complexes, inducing trunk ataxia. Whether the p.Glu841fs29* variation also affects receptor functioning has not yet been determined.

Finally, we discovered a missense variation, c.3007C>T, p.Arg1003Trp (reported once in the ExAC browser), affecting a highly conserved amino acid, in 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. To investigate the consequences of the p.Arg1003Trp variant, corresponding to p.Arg975Trp in rat *Grin3b,* we investigated the activity of receptor complexes including GluN1 and wild type or mutant GluN3B in Xenopus Oocytes. The p.Arg975Trp variant did not affect the glycine potency or Mg2+ block of recombinant GluN1/GluN3B receptors (Supplementary Figure 2A and B), indicative of normal receptor activity. Furthermore, we investigated the cell surface expression of GluN1/GluN3B-WT and GluN1/GluN3B-Arg975Trp receptor complexes in HEK293T cells. Immunocytochemistry showed reduced cell surface expression of GluN1/GluN3B-Arg975Trp compared to GluN1/GluN3B-WT (Supplementary Figure 2C), which may be caused by impaired intracellular trafficking. Notably, benign null alleles of *GRIN3B* have been reported, but were not associated with motor neuron disease,[10] suggesting that dominant negative mutations rather than haploinsufficiency of *GRIN3B* may lead to disease. Whether GluN3B-Arg975Trp exhibits dominant negative effects over GluN3B-WT remains unknown.

In summary, we have identified the first mutations in *GRIN3B* and *GRIK1* that link to ataxic phenotypes and/or intellectual disability. Two of the three cases in this work suffer from both motor dysfunction and intellectual disability, suggesting that glutamate signaling may be a shared mechanism connecting these two disorders. Additionally, we postulate that regular SCA diagnostics are occasionally requested to exclude cerebellar ataxia rather than confirm it, and these patients could benefit from a more restricted clinical classification of this disorder. 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 including genes coding for glutamatergic components.

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Conflict of interest

The authors declare they have no conflict of interest.

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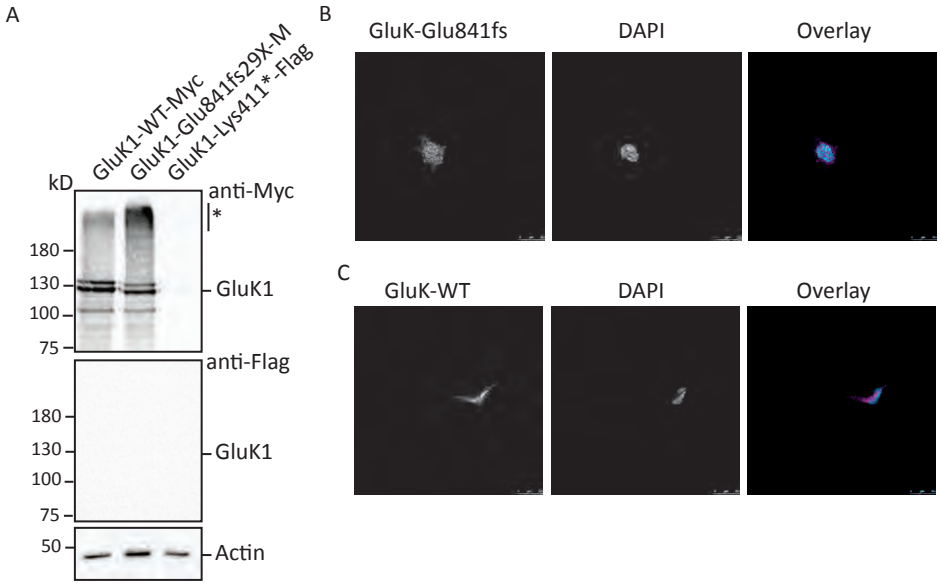
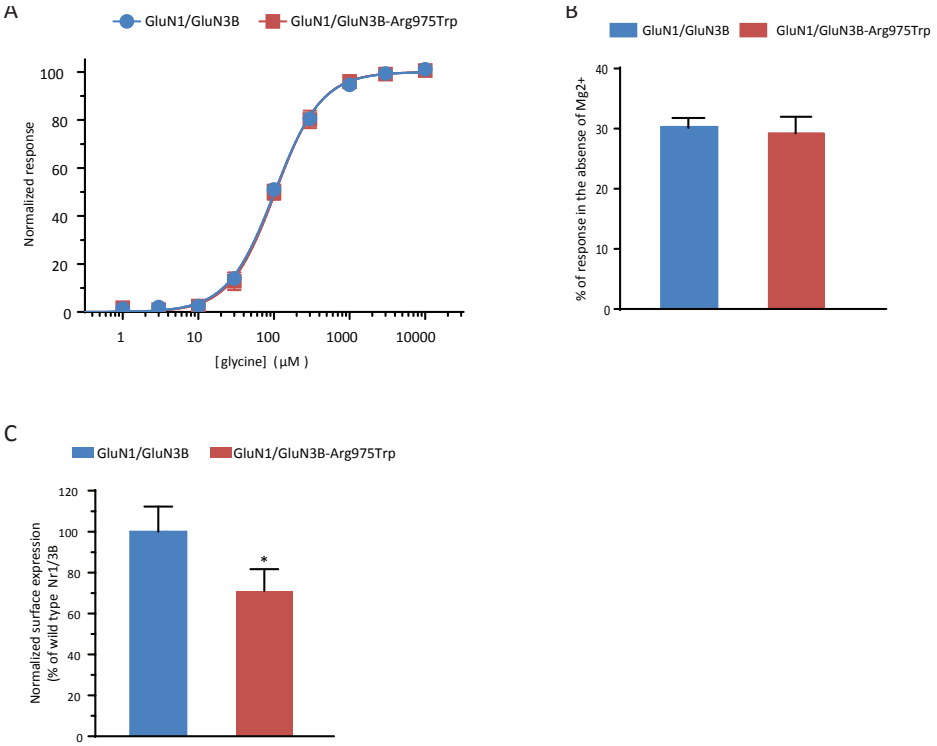
Supplementary Table 1. Genes tested by TRS

KEGG category	Gene symbol	Gene name	HGNC
SN1	*SLC38A3*	solute carrier family 38, member 3	18044
EAAT	*SLC1A3*	solute carrier family 1 (glial high affinity glutamate transporter), member 3	10941
	SLC1A2	solute carrier family 1 (glial high affinity glutamate transporter), member 2	10940
	SLC1A1	solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1	10939
	SLC1A6	solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6	10944
GLNT	*SLC38A1*	solute carrier family 38, member 1	13447
	SLC38A2	solute carrier family 38, member 2	13448
mGluR	*GRM2*	glutamate receptor, metabotropic 2	4594
	GRM3	glutamate receptor, metabotropic 3	4595
	GRM4	glutamate receptor, metabotropic 4	4596
	GRM5	glutamate receptor, metabotropic 5	4597
	GRM1	glutamate receptor, metabotropic 1	4593
	GRM7	glutamate receptor, metabotropic 7	4599
	GRM8	glutamate receptor, metabotropic 8	4600
GIRK	*KCNJ3*	potassium channel, inwardly rectifying subfamily J, member 3	6264
KA	*GRIK1*	glutamate receptor, ionotropic, kainate 1	4579
	GRIK2	glutamate receptor, ionotropic, kainate 2	4580
	GRIK3	glutamate receptor, ionotropic, kainate 3	4581
	GRIK4	glutamate receptor, ionotropic, kainate 4	4582
	GRIK5	glutamate receptor, ionotropic, kainate 5	4583
GLS	*GLS*	glutaminase	4331
	GLS2	glutaminase 2 (liver, mitochondrial)	29570
vGLUT	*SLC17A6*	solute carrier family 17 (vesicular glutamate transporter), member 6	16703
	SLC17A7	solute carrier family 17 (vesicular glutamate transporter), member 7	16704
	SLC17A8	solute carrier family 17 (vesicular glutamate transporter), member 8	20151
AMPA	*GRIA1*	glutamate receptor, ionotropic, AMPA 1	4571
	GRIA2	glutamate receptor, ionotropic, AMPA 2	4572
	GRIA3	glutamate receptor, ionotropic, AMPA 3	4573
	GRIA4	glutamate receptor, ionotropic, AMPA 4	4574
NMDAR	*GRIN1*	glutamate receptor, ionotropic, N-methyl D-aspartate 1	4584
	GRIN2A	glutamate receptor, ionotropic, N-methyl D-aspartate 2A	4585
	GRIN2B	glutamate receptor, ionotropic, N-methyl D-aspartate 2B	4586
	GRIN2C	glutamate receptor, ionotropic, N-methyl D-aspartate 2C	4587
	GRIN2D	glutamate receptor, ionotropic, N-methyl D-aspartate 2D	4588
	GRIN3A	glutamate receptor, ionotropic, N-methyl-D-aspartate 3A	16767
	GRIN3B	glutamate receptor, ionotropic, N-methyl-D-aspartate 3B	16768
TRPC1	*TRPC1*	transient receptor potential cation channel, subfamily C, member 1	12333
	TRPC3	transient receptor potential cation channel, subfamily C, member 3	12335
Homer	*HOMER1*	homer scaffolding protein 1	17512



Supplementary figure 1. *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 (B). GluK1-WT displayed robust surface expression (C). Scale bars, 25µm.



Supplementary figure 2. 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 described.[11] 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 Mg^{2+} is shown as the percentage of the response in absence of Mg^{2+} (cells voltage-clamped at -80 mV). The Grin3b- Arg975Trp variation did not alter the Mg^{2+} 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.
* $p < 0.05$