

LETTER TO THE EDITOR

Reply: A homozygous *GDAP2* loss-of-function variant in a patient with adult-onset cerebellar ataxia; and Novel *GDAP2* pathogenic variants cause autosomal recessive spinocerebellar ataxia-27 (SCAR27) in a Chinese family

Ilse Eidhof,¹ Jonathan Baets,^{2,3,4} Erik-Jan Kamsteeg,¹ Annette Schenck¹ and Bart P. van de Warrenburg⁵

- 1 Department of Human Genetics, Donders Institute for Brain, Cognition, and Behavior, Radboud University Medical Centre, 6525 GA Nijmegen, The Netherlands
- 2 Center for Molecular Neurology, University of Antwerp, 2610 Antwerp, Belgium
- 3 Institute Born-Bunge, University of Antwerp, 2610 Antwerp, Belgium
- 4 Neuromuscular Reference Centre, Department of Neurology, Antwerp University Hospital, 6520 Antwerp, Belgium
- 5 Department of Neurology, Donders Institute for Brain, Cognition, and Behavior, Radboud University Medical Centre, 6525 GC Nijmegen, The Netherlands

Correspondence to: Bart P. van de Warrenburg
Department of Neurology, Donders Institute for Brain, Cognition, and Behavior, Radboud University Medical Centre, 6525 GC Nijmegen, The Netherlands
E-mail: Bart.vandeWarrenburg@radboudumc.nl

Sir,

We read with great interest, the letters from Breza *et al.* (2020) and Dong *et al.* (2020). Following our recent publication in this journal reporting a new autosomal recessive cerebellar ataxia (ARCA) subtype caused by mutations in *GDAP2* (Eidhof *et al.*, 2018a), both groups each identified a patient with gene-disruptive mutations in the *GDAP2* gene by exome sequencing or after reanalysis of exome data.

First of all, these two independent reports are an important confirmation of our finding that bi-allelic *GDAP2* mutations cause cerebellar ataxia. Second, although Breza *et al.* (2020) have not provided further details on the size of the ataxia cohort they screened, the fact that both they and Dong *et al.* (2020) in their cohort of 60 unsolved ARCA patients, identified only a single patient with mutations in *GDAP2* suggests that this is a rare cause of ARCA. This is in line with our impression, as the two independent patients reported by us came from two large ataxia cohorts. Third, although the current number of patients is limited, there is so far a striking homogeneity in terms of phenotype: an adult-onset, slowly progressive cerebellar syndrome manifesting in the fourth decade of life, accompanied by pyramidal features/spasticity, and—in three of the currently four patients—cognitive decline. Whether the observed emotional

liability, aggressive behaviour, and depressive episodes in the patient reported by Breza *et al.* are also linked to *GDAP2* remains uncertain at this stage.

The homozygous frameshift variant, a 1-bp deletion, identified by Breza *et al.* (p.Pro45LeufsTer22), is located in exon 2 of the *GDAP2* gene and severely affects the *GDAP2* open reading frame. The variant damages the Macro domain and leads to complete absence of the CRAL-TRIO domain. Because of the presence of an early premature termination codon located 22 amino acids downstream of the variant, the mutated *GDAP2* transcript is likely targeted for nonsense-mediated decay (NMD). Thus, similar to the *GDAP2* variants that we described previously, this variant likely leads to loss of *GDAP2* protein levels.

The biallelic variants (p.Arg253* and p.Thr422fs*7) identified in the patient described by Dong *et al.* are located in exon 7 and exon 13 of the *GDAP2* gene. Both variants were predicted to be targeted for NMD or result in transcripts that encode prematurely terminated *GDAP2* proteins that either completely lack or contain a truncated CRAL-TRIO domain. Based on the expression of these variants using copy-DNA constructs, Dong *et al.* suggested that both p.Arg253* and p.Thr422fs*7 do not result in a loss of, but in truncated *GDAP2* proteins. However, there is a design problem with these experiments. NMD is triggered by

exon-junction complexes that bind the mRNA during the splicing process (Kervestin and Jacobson, 2012). By using copy-DNA, i.e. DNA without introns, the splicing step is eliminated and hence the utilized approach is not suitable to argue against NMD. It is therefore very likely that GDAP2 levels in the patient with the p.Arg253* and p.Thr422fs*7 variants reported by Dong *et al.* are strongly decreased, and loss-of-function is underlying the consistent phenotype. Dong *et al.* suggested that the CRAL-TRIO domain might be a mutational hotspot, and that loss of this domain, either via overall loss of the GDAP2 protein or a truncated GDAP2 protein that lacks a functional CRAL-TRIO domain, can be causative of ARCA. The GDAP2 CRAL-TRIO domain is indeed either directly or indirectly affected in all four cases. Nonetheless, more research will be required to understand the exact pathophysiological mechanisms.

Dong *et al.* hint towards the possibility that truncated GDAP2 mutations are associated with a less severe phenotype in comparison with mutations that lead to loss of GDAP2. However, current data suggest a dynamic phenotype based on disease duration, rather than a genotype-phenotype correlation, and, as stated earlier, compelling evidence for a mechanism involving a truncated GDAP2 protein rather than GDAP2 loss-of-function is missing.

It is currently unclear how loss of GDAP2 leads to progressive cerebellar degeneration. We previously showed that ARCA-associated genes have enriched connectivity at the protein level, and are strongly enriched for defective cellular stress responses and DNA repair, to which the cerebellum seems preferentially vulnerable (Eidhof *et al.*, 2018b, 2019). Based on its protein domains, GDAP2 connects to other ARCA-associated proteins implicated in cellular stress responses (Warde-Farley *et al.*, 2010; Eidhof *et al.*, 2018a, 2019). Indeed, Gdap2 expression levels in *Drosophila* respond to cellular stress and induction of cellular stress in *Drosophila* GDAP2 models severely reduced lifespan (Eidhof *et al.*, 2018a), supporting the possibility that GDAP2 acts as a metabolic sensor in the cerebellum that is required for an appropriate, effective response to stress. The exact role of GDAP2, however, needs further investigation.

Others have given this ARCA subtype caused by GDAP2 mutations the designation ARCA27 or SCAR27. However, we are in favour of using the recently proposed classification system for genetic movement disorders, including ataxia (Rossi *et al.*, 2018), and would like this entity to be referred to as ATX-GDAP2.

In conclusion, Breza *et al.* and Dong *et al.* have independently confirmed the causality of GDAP2 mutations in rare, late-onset ARCA. Despite the phenotypic homogeneity suggested so far, additional cases and a longer follow-up time will help us to delineate the full phenotypic GDAP2 spectrum. More research will be required to understand the biological function of GDAP2, the pathophysiological

consequences of GDAP2 mutations, and how these contribute to cerebellar degeneration and ataxia.

Data availability

Data availability is not applicable to this article as no new data were created or analysed in this study.

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Competing interests

The authors report no competing interests.

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