

# Biallelic *DAB1* Variants Are Associated With Mild Lissencephaly and Cerebellar Hypoplasia

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## Abstract

### Objective

We aimed to identify pathogenic variants in a girl with epilepsy, developmental delay, cerebellar ataxia, oral motor difficulty, and structural brain abnormalities with the use of whole-exome sequencing.

### Methods

Whole-exome trio analysis and molecular functional studies were performed in addition to the clinical findings and neuroimaging studies.

### Results

Brain MRI showed mild pachygyria, hypoplasia of the cerebellar vermis, and abnormal foliation of the cerebellar vermis, suspected for a variant in one of the genes of the Reelin pathway. Trio whole-exome sequencing and additional functional studies were performed to identify the pathogenic variants. Trio whole-exome sequencing revealed compound heterozygous splice variants in *DAB1*, both affecting the highly conserved functional phosphotyrosine-binding domain. Expression studies in patient-derived cells showed loss of normal transcripts, confirming pathogenicity.

### Conclusions

We conclude that these variants are very likely causally related to the cerebral phenotype and propose to consider loss-of-function *DAB1* variants in patients with RELN-like cortical malformations.

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## Glossary

**GoF** = gain of function; **LoF** = loss of function; **PTB** = phosphotyrosine binding; **WES** = whole-exome sequencing.

The Disabled-1 (*DAB1*) gene encodes a key regulator in Reelin signaling, a critical pathway mediating correct positioning of neurons within the developing brain.<sup>1,2</sup> In mice, both *Dab1* and *Reln* are essential for proper cortical layering during embryonic development. Binding of Reelin to the lipoprotein receptors *VLDLR* and *APOER2* on the neuronal surface leads to phosphorylation of *DAB1* and activates downstream signaling cascades. *Dab1*-depleted mice present with a phenotype comparable to Reelin-deficient mice, including disruption of neuronal layering in the cerebral cortex, hippocampus, and cerebellum.<sup>3</sup>

Yet, human loss-of-function (LoF) mutations in *DAB1* have not been described, whereas biallelic LoF mutations in *RELN* (OMIM #600514) are well known to cause a similar phenotype as seen in the murine counterpart. Recessive *RELN* variants cause a distinctive lissencephaly, associated with prominent hypoplasia of the pons, the cerebellar hemispheres, and the vermis.<sup>4</sup> A similar but milder phenotype is described for *VLDLR* (OMIM #192977) variants.<sup>5</sup> The only human disease related to *DAB1* is spinocerebellar ataxia type-37 (SCA37, OMIM #615945), caused by (ATTTC)<sub>n</sub> insertions in the 5'UTR of *DAB1*.<sup>6</sup> Several studies show that SCA37 occurs through gain-of-function (GoF) mechanisms, of which only 1 is directly related to *DAB1* expression because the insertion results in overexpression of *DAB1* protein and alternative *DAB1* transcripts.<sup>7</sup>

Here, we report a patient with biallelic LoF variants in *DAB1*, presenting with *RELN*-like malformations including mild lissencephaly and cerebellar hypoplasia.

## Methods

### Consent

The study was approved by the local IRBs (Erasmus MC Rotterdam, protocol METC-2012387). Written informed consent to participate in this study was obtained from the parents of the participant.

### Whole-Exome Sequencing

Whole-exome sequencing (WES) was performed on the Agilent Sure Select platform (Clinical research Exome Capture), run on HiSeq (101bp paired-end, Illumina), using the diagnostic certified pipeline of the department of Clinical Genetics, ErasmusMC, Rotterdam. The average coverage is ~50×. Data are demultiplexed by the Illumina Software CASAVA. Reads are mapped with the program BWA (bio-bwa.sourceforge.net/). Variants are detected with the Genome Analysis Toolkit (broadinstitute.org/gatk/). The Variant Calling File is filtered in Alissa Interpret.

### Sanger Sequencing

Amplification reactions were conducted according to standard methods and purified with ExoSAP-IT (USB). Direct sequencing was performed with Big Dye Terminator chemistry (Applied Biosystems). DNA fragment analysis was performed with capillary electrophoresis on an ABI3130 Genetic Analyzer (Applied Biosystems) with the software package Seqscape (Applied Biosystems).

### Quantitative Reverse Transcription PCR

Fibroblasts from skin biopsies were grown in DMEM (10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin) at 37°C and 5% CO<sub>2</sub>, followed by RNA isolation using the RNeasy mini kit (QIAGEN). RNA was reverse transcribed with the iScript cDNA synthesis kit (Bio-Rad Laboratories). Quantitative reverse transcription PCR was performed using iTaq Universal SYBR Green Supermix (Bio-Rad) and the following primer sequences: *DAB1*\_rt\_c307\_1F:TCGGGATTGATGAAGTTTCC;*DAB1*\_rt\_c307\_1R:AGCCTCAAACACAATGTACTGG;*DAB1*\_rt\_c67\_2F:GAGGATGCTCTGGGCTAGG;*DAB1*\_rt\_c67\_2R:AAAGATTTTGATTCTCCCAAAGG.

### Data Availability

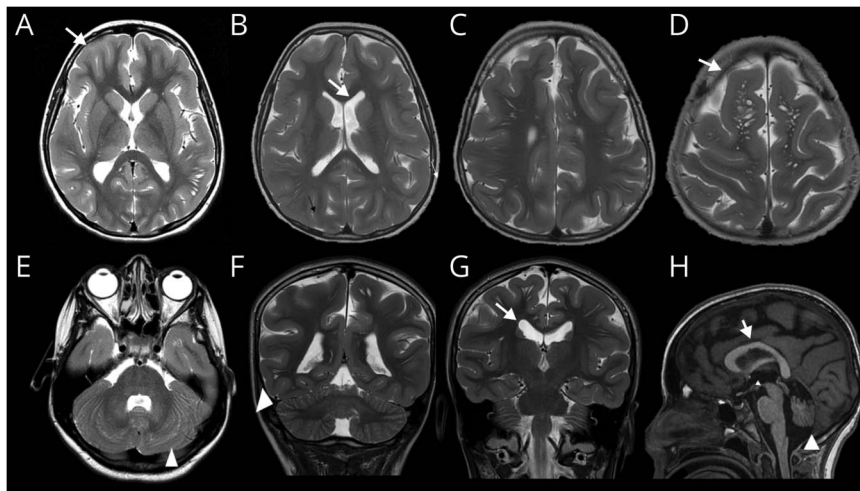
WES data are deposited at the ISO certified diagnostic laboratory of the Department of Clinical Genetics, Erasmus MC, in respect to the family's privacy.

## Results

### Case Report

The affected individual was born at term after an uneventful delivery from unrelated healthy parents. In infancy, she had gastrointestinal reflux and excessive crying. She tolled over at 8 months, sat unsupported at 14 months, and walked at age 3 years. Cognitive development initially raised no concern, but during the first years, learning problems became apparent and she now attends special school (IQ: 50–60). The onset of focal epilepsy was at age 6 years; seizure semiology was loss of awareness, staring, without clear motor signs. Oxcarbazepine (8 mg/d) reduced seizure frequency, but absences persist once/twice a week without additional signs. Physical examination at age 11 years showed mild cerebellar ataxia, oculomotor apraxia, mild dysmetria of the upper extremities, impaired tandem gait, impaired facial muscle coordination, dysarthria, instability during Romberg test, dysdiadokokinesis, squint, mild pyramidal signs, joint hypermobility, normal muscular tone, and symmetrically low deep tendon reflexes. Head circumference was –1 SD; weight and height were within the normal range. Standard EEG at age 10 years showed no epileptic activity, normal posterior activity, excess of theta and delta waves in frontopolar,

**Figure 1** Brain MRI



Brain MRI of the affected individual with axial T2-weighted images (A–E), coronal T2-weighted images (F and G), and midsagittal T1-weighted image (H). Mild and diffuse cortical pachygyria more prominent in the frontal lobes (arrow in A and D), mildly thin corpus callosum (H, arrow), hypoplasia and abnormal foliation of cerebellar hemispheres (E and F, arrow head) and more pronounced vermis hypoplasia (H, arrow head), enlarged perivascular spaces (A–G), and lateral ventricles (B and G, arrow), all reminiscent of an *RELN/VLDLR* pattern.

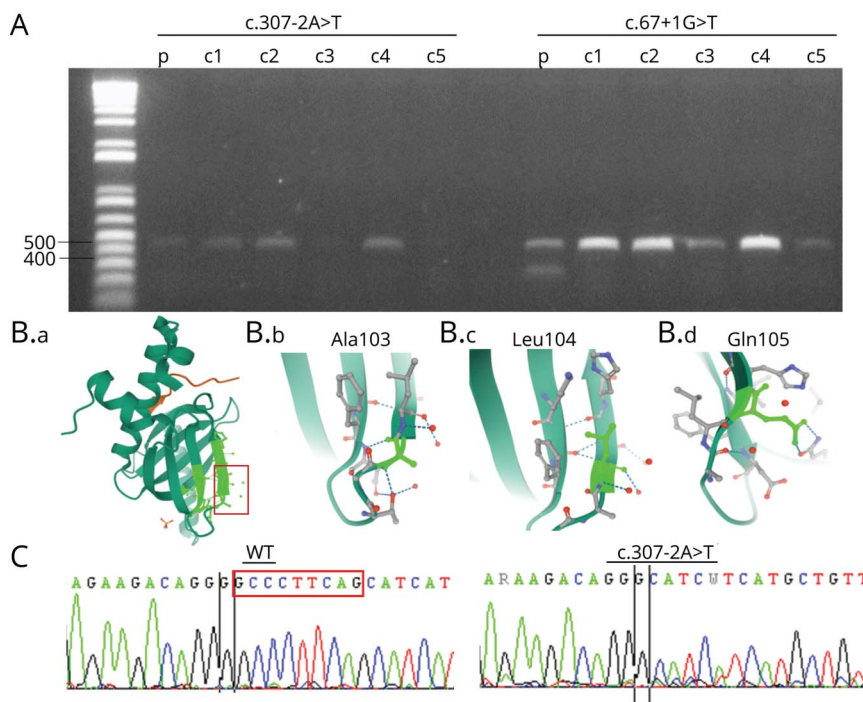
frontal, and temporal areas with occasional sharp waves in frontotemporal regions (left more than right), and normal photic stimulation response. Brain MRI at age 12 years showed cortical malformations reminiscent of *RELN*-related malformations, including mild pachygyria, i.e., decreased number of gyri with moderately thickened cortex (more prominent in the frontal lobes), mildly thin corpus callosum, enlarged perivascular spaces, and mildly enlarged lateral ventricles. The cerebellar vermis was hypoplastic and showed abnormal foliation.

Abnormal foliation was observed to a lesser extent in the cerebellar hemispheres. The pons, the basal ganglia, and the hippocampal folding were normal (figure 1).

### Genomic Analysis and Expression Studies

High-resolution genomic microarrays showed normal female pattern. Sanger sequencing of *RELN* was normal. WES trio analysis identified compound heterozygosity for *DAB1* splice site variants. The first variant (Chr1(GRCh37):g.57756635C>A

**Figure 2** Functional Analysis of *DAB1* Variants



(A) RT-PCR of *DAB1* mRNA from the affected individual(p) and 5 age- and sex-matched control samples(c1-5). Primers were designed to amplify a product of 450 bp for the 67+1G>T variant and a product of 470 bp for the 307-2A>T variant. For the 67+1G>T variant, an alternative mRNA splice product is formed in the affected individual, which could be explained by the deletion of exon 4 (exon 4 contains 203 bp). (B) Structural model of the *DAB1* PTB domain. The panel (B.a) shows the structure of the entire domain. Localization of the deleted amino acids is depicted in the other panels (B.b–B.d). (C) Sanger sequencing results of the c.307-2A>T transcript. PTB = phosphotyrosine binding; RT-PCR = reverse transcription PCR.

NM\_021080.3 c.67+1G>T, p.?) is located in the splice donor site of intron 4 and has never been reported in GnomAD. Splice prediction programs (MaxEntScan, NNSPLICE, GeneSplicer) predict an in-frame deletion of exon 4. Of interest, this deletion eliminates the ATG initiation site and the corresponding Kozak consensus sequence. Reverse transcription PCR on cDNA-derived from fibroblasts confirmed that the c.67+1G>T variant leads to a shorter, but stable, transcript (figure 2A). The second variant (Chr1(GRCh37):g.57538089T>A NM\_021080.3 c.307-2A>T, r.307\_315del9 p.Ala103\_Gln105del) affects the splice acceptor site of intron 6, resulting in an in-frame deletion of 3 amino acids of exon 7, which are part of a  $\beta$ -sheet forming the highly conserved phosphotyrosine-binding (PTB) domain (figure 2B). Sanger sequencing confirmed this deletion (figure 2C). Heterozygosity was confirmed for both parents. Despite database searches (genematcher.org) and international contacts (Neuro-MIG), we did not identify another individual with a similar phenotype.

## Discussion

Here, we report an individual with biallelic splice variants in *DAB1*. Given the RELN-like phenotype at MRI and the similarities of our patient with RELN/VLDRL-associated phenotypes, we conclude that the observed *DAB1* variants are very likely related to the cerebral malformations in our patient.<sup>4,5</sup> The *DAB1* splice variants in our patient result in alternative transcripts affecting the highly conserved PTB domain. Translation of any *DAB1* isoform containing this domain from the c.67+1G>T transcript is unlikely because it eliminates the methionine start codon. The c.307-2A>Tp.Ala103\_Gln105del variant results in a protein containing this domain, but with a deletion of 3 amino acids, most likely altering protein folding. Although the precise effect of this deletion on protein structure and binding capacities remains unclear, the heterozygote parent carrying the c.67+1G>T p.? variant is healthy, supporting the additional pathogenic effect of the c.307-2A>Tp.Ala103\_Gln105del variant.

In vitro, the PTB domain binds to cytoplasmic tails of the VLDLR and apoER2. This interaction is essential because binding of Reelin to these receptors induces *DAB1* tyrosine phosphorylation and subsequent activation of downstream signaling pathways. Mice lacking the *DAB1* PTB domain show almost complete absence of distinct cell layers in the cortex, a small and unfoliated cerebellum, and abnormal neuronal layering in the hippocampus.<sup>3</sup>

GoF mechanisms have been previously described in relation to *DAB1* autosomal dominant mutations, causing SCA37.<sup>6</sup> Although our patient presents with mild cerebellar ataxia, most of the phenotypic features are very distinguishable from SCA37.<sup>7,8</sup> The proposed mechanisms causing SCA37 (e.g., *DAB1* overexpression, RNA foci formation) are very distinct from the effect of the LoF variants described here, which

explains the phenotypic differences and the early age at onset in our patient. Our results indicate that *DAB1* LoF variants should be considered in patients with RELN-like cortical malformations at MRI. In addition, we propose inclusion of *DAB1* in diagnostic exome panels devoted to brain malformations, intellectual disability, and epilepsy.

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## Disclosure

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## Appendix Authors

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<b>Martina Wilke, PhD</b>	ErasmusMC University Medical Center, Rotterdam, the Netherlands	Analysis of data and revising
<b>Marjon van Slegtenhorst, PhD</b>	ErasmusMC University Medical Center, Rotterdam, the Netherlands	Analysis of data and revising
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<b>Marjolein H.G. Dremmen, MD, MSc</b>	ErasmusMC University Medical Center, Rotterdam, the Netherlands	Analysis of data and revising
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<b>A. James Barkovich, MD, PhD</b>	University of California, San Francisco	Supervision, interpretation of data, and revising
<b>Grazia M.S. Mancini, MD, PhD</b>	ErasmusMC University Medical Center, Rotterdam, the Netherlands	Supervision, formulation of research goals, interpretation of data, and writing the draft

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