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LETTER TO THE EDITOR

Pontocerebellar hypoplasia with spinal muscular atrophy (PCH1): identification of *SLC25A46* mutations in the original Dutch PCH1 family

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Sir,

Recently, Wan *et al.* (2016) described mutations in *SLC25A46* in patients with lethal congenital pontocerebellar hypoplasia (PCH). PCH is a rare, heterogeneous prenatal onset neurodegenerative disorder, mainly but not exclusively affecting cerebellum and pons. Based on clinical features and genetic causes, the current classification comprises 10 distinct PCH subtypes. Concurrent with the report by Wan *et al.*, other studies have identified *SLC25A46* mutations in optic atrophy spectrum disorders including other neurological features such as axonal Charcot-Marie-Tooth (CMT) neuropathy and/or cerebellar atrophy (Abrams *et al.*, 2015; Nguyen *et al.*, 2017).

The patients described by Wan *et al.* (2016) presented with PCH, optic atrophy and apnoea and showed neuromuscular involvement with evidence of axonal sensorimotor neuropathy. These findings were compatible with PCH type 1 (PCH1), which is characterized by the combination of spinal muscular atrophy and PCH (Barth, 1993). However, no autopsy studies were available to confirm coincident degeneration of spinal motor neurons (Wan *et al.*, 2016).

Following earlier reports of similar cases (Norman, 1961; Goutiéres *et al.*, 1977; Chou *et al.*, 1990; Kamoshita *et al.*, 1990), Barth defined PCH1 as a clinically distinct type of PCH, based on the presence of anterior horn cell degeneration in addition to PCH (Barth, 1993). Barth described a family with three affected siblings born to non-consanguineous Dutch parents (Barth, 1992, 1993). All three children were delivered after full-term pregnancy and died within 1 day after birth due to lack of spontaneous respiration and profound muscle weakness. Prenatal ultrasound detected no foetal abnormalities; only the second pregnancy was complicated by polyhydramnios. Autopsy on the second child, a girl, showed a very small cerebellum, degenerative changes in the inferior olive nucleus; the spinal cord was not available for pathological diagnosis. The third child, a boy, had multiple congenital contractures, severe hypotonia and convulsions. A CT scan revealed severe PCH (Supplementary Fig. 1). Ophthalmological examination showed a pale optic disc. Autopsy confirmed olivopontocerebellar degeneration, similar to the deceased sister. Histological analysis of the cervical spinal cord showed loss and ongoing degeneration of spinal motor neurons in the medial parts of the anterior horn at all levels from the cervical to the lumbar region. A biopsy from the rectus femoris muscle showed severely atrophied muscle fibres of all types without type grouping. (Fig. 1A–D) (Barth, 1993).

In 2012, mutations in *EXOSC3* were identified as an important cause of PCH1 (Wan *et al.*, 2012). However,

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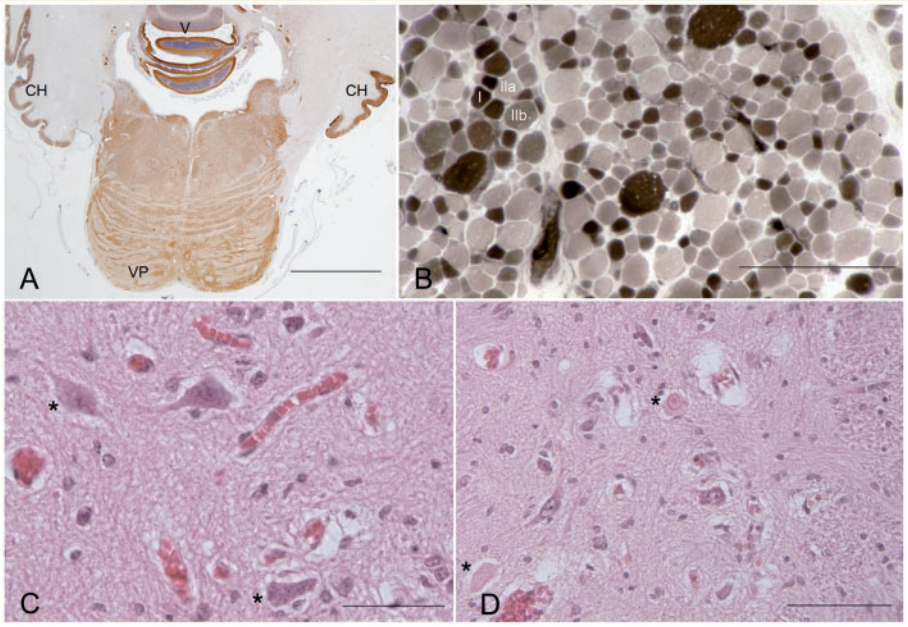

Figure 1 Neuropathology showing pontocerebellar hypoplasia, muscle atrophy and spinal motor neuron degeneration in the third affected sibling. (**A**) Transverse section of cerebellum and pons at the level of the vestibular nuclear complex. The hemispheric cerebellar cortex has underdeveloped folia with poor branching. The vermal cortex shows a mature aspect. The ventral pons is mildly hypoplastic with its height approximately equal to that of the tegmentum. Synaptophysin antibody staining. Scale bar = 5 mm. CH = cerebellar hemispheric cortex; V = cerebellar vermal cortex; VP = ventral pons. (**B**) Transverse section of frozen rectus femoris muscle, myosin ATPase pH 4.3. Random extreme fibre size variability and atrophy were noted, affecting type I as well as subtypes IIA and IIB fibres, with the smallest fibre diameters measuring $<5\,\mu m$ (in normal neonates, all types $>10\,\mu m$). Occasional very large type I fibres are normal at this age. Scale bar = 100 μm. (**C**) Anterior horn of cervical spinal cord, showing degenerating spinal motor neurons with loss of nuclear staining, indicated by asterisks, and gliosis. Haematoxylin and eosin stain. Scale bar = 50 μm. (**D**) Anterior horn of lumbar spinal cord, showing degenerated, shrunken motor neurons, and gliosis. Some lost neurons are represented by eosinophilic 'ghosts', indicated by asterisks. Haematoxylin and eosin stain. Scale bar = 100 μm.

mutations in this gene are generally associated with relatively milder forms of PCH1, without respiratory failure and some patients surviving into adulthood (Rudnik-Schöneborn *et al.*, 2013; Eggens *et al.*, 2014). In keeping with this, we observed no *EXOSC3* mutations in the Dutch PCH1 family reported by Barth (1992, 1993). We failed to identify biallelic variants in the known PCH genes or convincing co-segregating biallelic variants in any other gene by whole-exome sequencing of the DNA of the third affected child.

In view of the clinical similarities of the patients described by Wan *et al.* (2016) and the Dutch family, we reanalysed the whole exome sequencing data. This revealed a heterozygous mutation leading to a premature stop codon (c.691C>T | NM_138773; p.R231* | NP_620128) in exon 8 of the *SLC25A46* gene, but no additional truncating or missense *SLC25A46* mutation was detected. Analysis of patient fibroblast mRNA showed monoallelic expression of the *SLC25A46* allele with the stop mutation in exon 8, indicating loss of expression of the other allele. To explain this loss of expression, we proceeded with whole genome sequencing and detected a heterozygous deletion of ~2.4 kb encompassing exon 3 of the *SLC25A46* gene. Exon 3 contains 58 nucleotides and this deletion therefore results in a frameshift. A deletion-spanning PCR and subsequent sequencing of the PCR product confirmed a deletion of 2391 bases of genomic sequence. The stop mutation in exon 8 and the exon 3 deletion were located on different alleles and also present in a compound heterozygous state in the second affected child (Fig. 2A–C and Supplementary material). Immunoblot showed absence of SLC25A46 protein in patient fibroblasts, suggesting instability of the *SLC25A46* mRNA or its protein (Fig. 2D and Supplementary material).

SLC25A46 is located in the outer mitochondrial membrane and plays an important role in maintaining the mitochondrial cristae and balancing mitochondrial fission and fusion, probably acting in a pro-fission manner (Abrams *et al.*, 2015; Janer *et al.*, 2016). Assessment of mitochondrial membrane potential, as measured by accumulation of tetramethylrhodamine, methyl ester, perchlorate (TMRM), showed reduced uptake in the patient compared to control (Supplementary material and Supplementary Fig. 4). This indicates a decrease of mitochondrial membrane potential in the patient compared to the control.

Notably, other genes involved in mitochondrial dynamics are also associated with optic nerve atrophy, e.g. *OPA1*, mutated in autosomal dominant optic nerve atrophy, and *MFN2*, an important cause of axonal CMT, which can be accompanied by optic nerve atrophy. The neurological

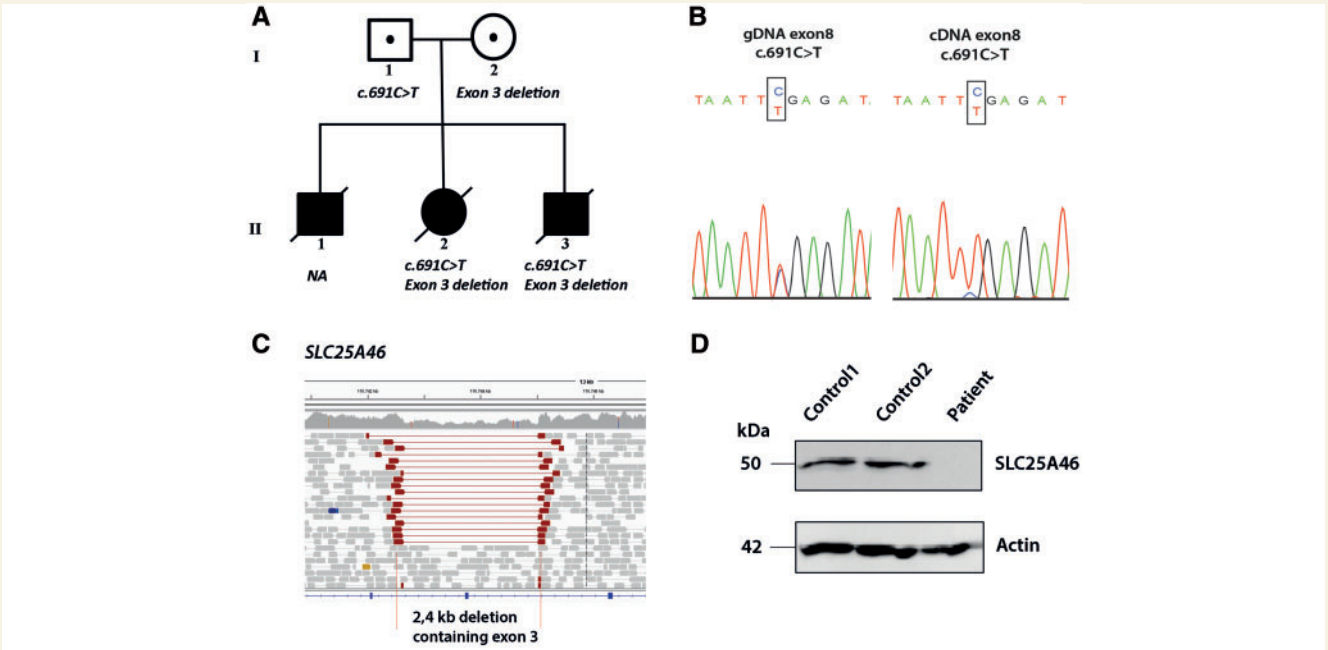

Figure 2 Pedigree of the Dutch PCH1 family, identified *SLC25A46* mutations and cDNA analysis. (**A**) Pedigree of the family. Black filled symbols = affected individuals. Dotted symbols = carrier status. NA = not analysed. Identified *SLC25A46* mutations are noted below each individual. No DNA was available of Patient II.1. (**B**) Sequence traces of *SLC25A46* exon 8 of Patient II.3 showing the paternally inherited c.691C > T (p.R231*) nonsense mutation in gDNA (*left*) and cDNA (*right*), indicating monoallelic expression of this allele. (**C**) Snapshot from Integrative Genomics Viewer showing a heterozygous deletion of about 2.4 kb of genomic DNA in Patient II.3. The deleted sequence contains exon 3 of the *SLC25A46* gene. (**D**) Western blot analysis of SLC25A46 in two controls compared to patient II.3, showing no SLC25A46 protein in the patient. Actin was used as an input control.

phenotypes caused by *SLC25A46* mutations are variable, but fit within this spectrum. Optic nerve pathology seems to be a consistent finding in *SLC25A46* related phenotypes, irrespective of disease severity. However, this feature might be overlooked in early fatal disease.

In essence, we identified the causal *SLC25A46* variants in the original Dutch family that was exemplary for the delineation of PCH1 as a distinct clinical subtype. We provide neuropathological evidence that loss of functional *SLC25A46* indeed causes motor neuron degeneration, categorizing *SLC25A46*-associated PCH as a subtype of PCH1. We suggest classifying *SLC25A46*-associated PCH1 as PCH1D (mutations in *VRK1*, *EXOSC3* and *EXOSC8* are associated with PCH1A, PCH1B and PCH1C, respectively). PCH1D is clinically distinguishable from other PCH1 subtypes by optic nerve involvement, respiratory failure and early death and is at the most severe end of the broad spectrum of *SLC25A46*-related conditions.

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Supplementary material

Supplementary material is available at *Brain* online.

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