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LETTER TO THE EDITOR

MATR3 haploinsufficiency and early-onset neurodegeneration

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As recently reviewed in *Brain*, the knowledge about the monogenetic spectrum underlying familial amyotrophic lateral sclerosis (ALS) has grown substantially in recent years.[1] To date, 34 different genes have been linked to ALS, including *SOD1*, *TARDBP*, *FUS*, *TBK1*, *SETX*, *ALS2*, *DCTN1*, *VAPB*, *CHMP2B*, *ANG*, *FIG4*, *ATXN2*, *SPG11*, *VCP*, *OPTN*, *SIGMAR1*, *C9orf72*, *UBQLN2*, *SQSTM1*, *PFN1*, *HNRNPA1*, *ERBB4*, *MATR3*, *TUBA4A*, *CHCHD10*, *KIF5A*, *ANXA11*, *TIA1*, *NEK1*, *C21orf2*, *MOBP*, *SCFD1*, *CCNF*, and *GYLD*.[1,2] Hypotheses regarding ALS development are numerous and comprise impaired mitochondrial function, dysregulation of protein degradation, disturbed axonal transport, alterations in autophagy, neuroinflammation, and aberrant regulation of cellular kinases. The shared end path of these pathomechanisms is selective motor neuron death leading to progressive muscle weakness, bulbar palsy, and respiratory failure (clinical subtypes of ALS are reviewed by Masrori and Van Damme[3]). To date, no effective treatment is available for affected individuals.

In 2014, Johnson *et al.*[4] identified variants in *MATR3* as a cause of familial ALS (ALS21, MIM #606070). *MATR3* encodes nuclear protein matrin 3 (MATR3), which constitutes an important component of the nuclear matrix. MATR3 has DNA and RNA-binding capacities and is involved in cell type-specific gene regulation, transcriptional regulation and DNA repair.[5] Clinical features of *MATR3*-asociated ALS include distal muscle weakness and bulbar signs, such as dysarthria and dysphagia. Some individuals show cognitive decline.[6] No association of *MATR3* with paediatric diseases has been reported so far.

Interestingly, Park *et al.*[7] described a 6-year-old male with a homozygous truncating variant in *SOD1*, leading to early-onset neurodegeneration with developmental disability, tetraspasticity, and hyperekplexia-like features. The clinical phenotype was distinctly different compared to the classical manifestation in familial ALS patients with *SOD1* mutations. This demonstrates that genes linked to ALS may be associated with allelic conditions and thus a broader clinical spectrum than previously thought.

Comparable to the findings of Park *et al.*,[7] we report here on a child with a heterozygous *de novo* variant in *MATR3*, causing an early-onset disease phenotype with severe neurodegeneration that is distinctly different from previous reports on *MATR3*-associated disease.

The study was performed according to the Declaration of Helsinki. Genetic and experimental procedures were undertaken after consent from the patient's parents was obtained. Written consent for the publication of photographs was obtained.

The patient was the second child of non-consanguineous parents with Romanian ancestry. A 6-year-old sister and a 1-year-old brother are healthy. He was born at term after an uneventful pregnancy (weight 3700 g, length 52 cm, head circumference according to the parents within normal range). After birth the patient showed muscular hypotonia and because of feeding difficulties, a nasogastric tube was placed. Epileptic seizures first occurred at the age of 2 months. Epilepsy was treated with valproate; however, without achieving seizure freedom. Brain MRI at the age of 4 months revealed widening of outer and inner CSF spaces. Diagnostic work-up including screening for metabolic diseases (amino acids in plasma, organic acids in urine, transferrin electrophoresis, analysis of CSF, purine and pyrimidine metabolites,

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analysis of very-long-chain fatty acid, etc.) was without specific findings. Genetic testing including chromosome analysis, microarray analysis and genetic panel diagnostics (*ALDH7A1*, *CDKL5*, *FLOR1*, *KCNQ2*, *POLG*, *SCN1A*, *SLC2A1*, *STXBP1* and *ARX*) was normal.

At the current age of 2 years the boy shows severe microcephaly (head circumference <1st percentile, Z-score –5.72) and profound developmental disability (Fig. 1A and B). He is unable to move independently and shows profound muscular hypotonia. He shows a hypotonic-dystonic movement pattern, and tendon reflexes are brisk. There is no speech development. Epilepsy is treated with levetiracetam und carbamazepine. Nevertheless, he is suffering from daily epileptic seizures (mostly short episodes with eye deviation and a duration of 20–30 s). EEG is pathological with high amplitude activity and predominantly left-sided epileptiform discharges. Brain MRI demonstrates supratentorial brain atrophy, delayed myelination and hypoplasia of the corpus callosum (Fig. 1C and D).

Whole-exome sequencing, performed on DNA from the child and both parents, failed to identify (likely) pathogenic variants in any genes previously associated with childhood-onset neurological disorders. The only suspicious finding was a *de novo* transition (NM_018834.6:c.1306G>A; hg19: chr5:138653408G>A) in exon 7 of *MATR3*, predictably leading to the substitution of a highly conserved amino acid residue (p.Glu436Lys). The missense variant was absent from more than 280 000 control alleles in gnomAD and more than 40 000 alleles from in-house sequenced individuals with various clinical conditions. *In silico* evaluation predicted a deleterious effect of the variant (CADD score of 23.2; PolyPhen-2 score of 0.99). Moreover, the site affected by the variant was expected to be intolerant against mutational changes and belongs to the 10% of most constraint coding regions of the genome (Supplementary Fig. 1).[8] Unlike ALS-linked *MATR3* missense variants predominantly affecting N- and C-terminal portions of the protein, c.1306G>A (p.Glu436Lys) mapped to the first of two central RNA-recognition motifs (RRM1; Fig. 1). We were unable to identify any further *MATR3 de novo* changes in whole-exome sequencing data from >31 000 parent-offspring trios assessed for paediatric disorders, indicating that *de novo* missense variants are an extraordinarily rare mutational event.[9] Notably, DECIPHER lists seven *de novo* deletions encompassing *MATR3* in subjects with phenotypes overlapping that of the described child here (developmental delay, muscular hypotonia, feeding difficulties, epilepsy), but there are no reported cases where *MATR3* alone has been deleted (https://decipher.sanger.ac.uk/; accessed 5 March 2021).

To investigate the functional consequences of the *MATR3* variant, we performed immunoblotting analysis of protein lysates derived from patient skin fibroblasts. In brief, cell culture was performed as described previously.[10] Subcellular fractionation was performed using the NE-PER™ Nuclear and Cytoplasmic Extraction Kit (Thermo Scientific #78835) according to the manufacturer's protocol. Primary antibodies against MATR3 (anti-rabbit; 1:1000; Proteintech #12202-2-AP), Lamin AC (anti-mouse, 1:5000; Thermo Scientific, SAB4200236) or GAPDH (anti-mouse, 1:4000; Thermo Scientific, AM4300) were used.

As depicted in Fig. 1F and G, we observed a significant reduction of ~50% of MATR3 protein levels in nuclear fractions compared to healthy control subjects. This observation supports a disease-

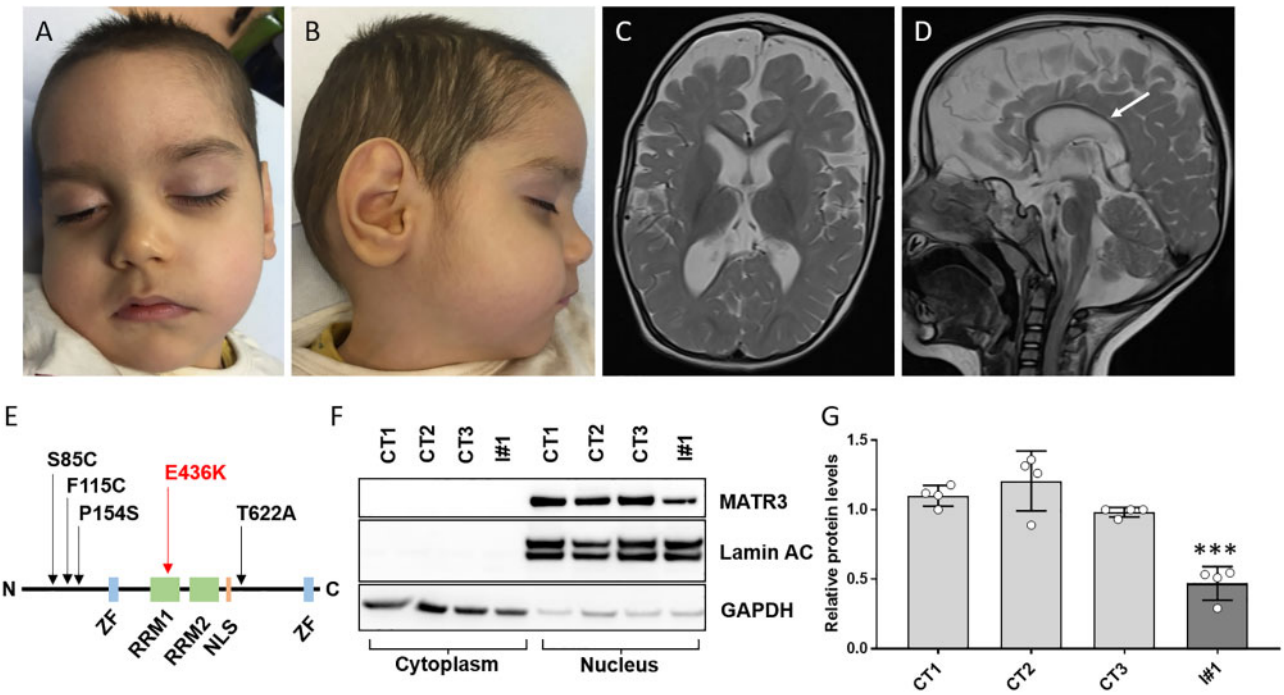

Figure 1 Clinical findings and functional consequences of MATR3 haploinsufficiency. (**A** and **B**) Clinical photographs of the affected individual at the age of 2 years showing microcephaly. (**C** and **D**) Brain MRI images at the age of 2 years (T_2-weighted sequences, axial and sagittal views) demonstrating cerebral atrophy with widening of external and inner CSF spaces. Myelination is delayed and corpus callosum is severely hypoplastic (white arrow). (**E**) Schematic domain representation of MATR3. ALS-linked variants are shown in black and the variant identified herein is shown in red. Only those ALS-linked variants are depicted which are listed as 'pathogenic' in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). NLS = nuclear localization signal; RRM1 = RNA-recognition motif 1; RRM2 = RNA-recognition motif 2; ZF = zinc finger motifs. (**F**) Representative immunoblot image depicting cytoplasmic and nuclear fractions of healthy controls (CT1-2 = age-matched male individuals, CT3 = age-matched female individual) and patient-derived (I#1) fibroblasts (the corresponding unmodified full-length blots can be found in the Supplementary material). (**G**) Quantitated density of immunoblot bands from four independent experiments. Average values are presented as the mean ± standard error of the mean (SEM); ***$P < 0.001$ significantly different from controls. In patient-derived fibroblasts, nuclear MATR3 amounts are clearly reduced.

relevant role for c.1306G>A (p.Glu436Lys) given that metrics from gnomAD predict that dosage reduction of MATR3 is not tolerated [probability of being loss-of-function intolerant score of 1.0; observed/expected (o/e) ratio = 0].

In addition to immunoblotting, we also performed MATR3 immunofluorescence imaging. As depicted in Supplementary Fig. 2, no obvious mislocalization or aggregation of MATR3 was detectable in patient-derived cells.

The findings reported above suggest a disease-causing role of the *MATR3* variant identified in this study via a haploinsufficiency mechanism and a potential extension of *MATR3*-associated allelic conditions. Moreover, our results further support the notion that alterations in ALS-associated genes may have a crucial impact on neuronal viability and function. However, paediatric cases as now reported for *SOD1* and *MATR3* additionally point to the relevance of these genes for early neurodevelopment. This broadens our view on the physiological function of these genes. Moreover, it might have important implications for ALS treatment approaches that are currently under development. Several of these strategies aim at lowering levels of specific gene products since most mutations that have been identified in familial ALS are thought to be disease-causing by gain-of-function mechanisms.[11] However, the reports of Park *et al.*,[7] as well as the case illustrated here, highlight the fact that both loss-of-function and gain-of-function mechanisms may have detrimental effects.

Data availability

The main data associated with this study are present in the article or Supplementary material. Additional raw data are available from the corresponding author, upon reasonable request.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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