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Short clinical report

Mutations in the mitochondrial gene *C12ORF65* lead to syndromic autosomal recessive intellectual disability and show genotype phenotype correlation

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ABSTRACT

Homozygosity mapping and exome sequencing in two affected siblings of a consanguineous family with mild intellectual disability, spastic paraplegia, and strabismus revealed a homozygous premature stop mutation at codon 139 of *C12ORF65*. Two previous studies reported truncating mutations at positions 84 and 132 of the protein. However, symptoms of the referred patients were only partially overlapping. Considering our findings, we now conclude that truncating mutations in *C12ORF65* lead to a variable phenotype with intellectual disability, spastic paraplegia, and ophthalmoplegia as common symptoms. Further, we confirm a genotype–phenotype correlation between increasing length of the truncated protein and decreasing severity of symptoms.

1. Introduction

C12ORF65 is a nuclear gene, which encodes a codon-independent translation release factor in mitochondria, and is probably crucial for the release of newly synthesized proteins from mitochondrial ribosomes [1]. Two studies reporting human mutations in *C12ORF65* have been published so far. Antonicka and colleagues identified two different homozygous mutations leading to the same premature stop at codon 84 in 3 patients presenting with intellectual disability, encephalomyopathy, optic atrophy, and ophthalmoplegia [2]. The stop codon lies within the RF-1 domain (Fig. 1) and is very close to the important GGQ motif of this domain [2]. The authors assumed that nonsense-mediated mRNA decay does not contribute to the loss of function of the *C12ORF65* gene product, because the level of *C12ORF65* mRNA was not significantly reduced in RT-PCR. Further examinations showed severe assembly defects in oxidative phosphorylation (OXPHOS) complexes I, IV and V in patients' fibroblasts, which could be rescued with expression of the wild-type *C12ORF65* cDNA. Cerebral MRI showed bilateral abnormalities [2]. Very recently, Shimazaki and colleagues reported on a truncated C12ORF65 protein at amino acid 132, which is also located within the RF-1 domain (Fig. 1), in patients with hereditary spastic paraplegia, optic atrophy, and peripheral neuropathy starting in the lower extremities. Electroencephalogram, electrocardiogram and brain computed tomogram showed no remarkable findings. Immunoblot analysis showed a truncated C12orf65 protein in patients' cell lines indicating that the mutated transcript escapes nonsense mediated mRNA decay. Cell lines of the patients also showed severe assembly defects in OXPHOS complexes I and IV, but only a milder decrease in complex V [3].

Here we report on a novel truncating mutation in two affected siblings with mild intellectual disability, spastic paraplegia, and strabismus. We delineate the symptom spectrum of truncating mutations in *C12ORF65* and describe a genotype–phenotype correlation.

2. Clinical report

This study was approved by the Ethics Committees of the University Bonn and of the University Erlangen-Nürnberg in Germany. Informed consent of all examined persons or of their guardians was obtained.

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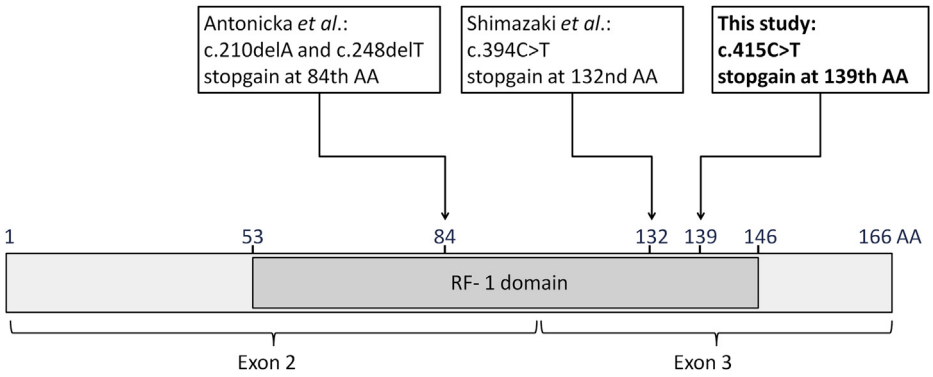

Fig. 1. Schematic structure of C12ORF65 that shows the exon Intron structure, the localization of the RF-1 domain and the positions of the identified mutations.

We examined two affected siblings, MR013-01 and MR013-02, who were presented at ages 27 and 24 years, respectively, with mild intellectual disability, strabismus that is probably due to ophthalmoplegia and a happy personality. Brain magnetic resonance imaging of MR013-02 showed no remarkable findings. The affected woman MR013-01 additionally showed facial features including low set eyebrows, hypertelorism, broad nasal bridge, thin upper lip, and round face (Fig. 2), had arthrogryposis of small joints of the upper and lower extremities, muscular weakness of the lower extremities and malposition of both feet. She lost her ability to walk without support in early adult life. Growth parameters and head circumferences of both affected siblings were unremarkable at birth and at the time of examination. Neither anomalies of the inner organs nor impairment of hearing and vision were noted. None of the patients showed epilepsy or tremor.

Interestingly, MR013-06, the maternal aunt of the siblings, also has consanguineous parents and presented with mild intellectual disability and weakness and malformation of the feet (Fig. 2).

Under the assumption that all three persons with intellectual disability would have the same mutation, genome wide mapping revealed one small (1.2 Mb) candidate region on chromosome 18 [4]. Sanger sequencing of all coding exons of the 10 RefSeq genes in this region revealed no candidate mutations. We thus considered that the differences of the phenotypes between the affected siblings and their aunt might reflect two different pathogenic entities, both segregating in the extended family. Re-evaluating the mapping data showed seven candidate regions of a total length of 90 Mb that segregated only with both affected siblings but not with their aunt.

We sequenced the affected male sibling MR013-02 on the SOLiD platform 5500 XL after enrichment of the exome with the 3rd version of Agilent SureSelect whole Exome kit. Then, after careful consideration of the conservation of the candidate mutations as well as of the results of four *in silico* prediction programs, we excluded the candidate mutations in ethnically matched healthy controls. Further details on sequencing and filtering steps can be obtained from previous publications [5,6]. We were left with a stop gain in exon 3 of *C12ORF65* that leads to a premature stop codon at p.Q139 within an RF-1 domain (Fig. 1). This candidate mutation was homozygous in both siblings MR013-01 and MR013-02 and heterozygous or absent in all other family members including the aunt MR013-06. Although no cell lines of the patients were available for further tests, we assume that the truncated protein is expressed, considering that even the 7 amino acid shorter truncated protein reported by Shimazaki and colleagues could be detected by Western blot.

Table 1
Summary of common symptoms of truncating mutations in *C12ORF65*.

	Antonicka	Shimazaki	This study
Length of truncated protein	84 AA	132 AA	139 AA
#Affected	3	2	2
Age at examination	8 y, 20 y, 22 y	42 y, 32 y	27 y, 24 y
Intellectual disability	Severe	Mild	Mild
Strabismus/ophthalmoplegia	+	+/−	+
Distinct facial features	−[a]	−	+
Brain abnormalities in MRI/CT	+/−/+	−	−
Weakness of distal muscles/SPG	+	+	+/−
Optic atrophy/loss of vision acuity	+	+	−
Early death	8 y, −, 22 y	−	−

[a] Personal communication with Elsebet Østergaard and Jan Smeitink.

3. Discussion

We consider the identified mutation in *C12ORF65* to be causative for the mild intellectual disability and the ophthalmoplegia in both affected siblings MR013-01 and MR013-02. The spastic paraplegia (SPG) symptoms of MR013-01 might also be explained by the identified mutation. Nevertheless, the brother MR013-02 does not have SPG while the aunt MR013-06 shows SPG-like symptoms though she does not carry the *C12ORF65* mutation. Under the assumption of another, yet not identified mutation that leads to SPG by MR013-01 and her aunt MR013-06 we undertook homozygosity mapping again and found one candidate region shared only by MR013-01 and the aunt, but not by MR013-02. The region is on chromosome 2 between 4.19 and 8.04 Mb and contains 4 coding RefSeq genes, *SOX11*, *CMPK2*, *RSAD1*, and *RNF144A*. Sequencing the coding exon of *SOX11*, a gene that is mainly expressed in the nervous system and is probably important in the developing nervous system, did not show any genetic changes. The available data on the other three genes do not indicate an obvious link to the phenotype or to neurological functions. Thus, based on the clinical and genetic data, we strongly suggest that the mutation in *C12ORF65* is causative for all the symptoms of the siblings including the SPG symptoms in MR013-01, even though we cannot fully exclude the existence of another mutation, that we did not detect because it is not covered by the NGS or because it is in a regulatory element. The absence of SPG in MR013-02 may be due to phenotype variability. It is also possible that this symptom becomes manifest at a later age. In our opinion, another independent mutation probably causes the aunt's phenotype. Such an occurrence of multiple recessive disorders in one family is not surprising in pedigrees with multiple consanguineous marriages [5].

Antonicka and colleagues reported that a premature stop at codon 84 of *C12ORF65* leads to intellectual disability, encephalomyopathy, optic atrophy, and ophthalmoplegia [2]. Furthermore, cerebral MRI showed bilateral abnormalities and the patients