LETTER TO THE EDITOR

‘Behr syndrome’ with OPA1 compound heterozygote mutations

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Sir,

We have been following with great interest the developments in the field of phenotypic diversity associated with mutations in the OPA1 gene, having contributed to describe the DOA ‘plus’ phenotype in a joint effort with other groups (Amati-Bonneau et al., 2008; Hudson et al., 2008; Yu-Wai-Mann et al., 2010). A developing story concerns the increasingly recognized cases associated with OPA1 mutations presenting with a childhood onset syndrome combining optic atrophy with spastic paraplegia, cerebellar ataxia and possibly other neurological features (Yu-Wai-Mann et al., 2010; Marelli et al., 2011; Pretegiani et al., 2011; Schaaf et al., 2011). This phenotype fits the description of Behr in 1909, who presented a series of cases of ‘complicated familial optic atrophy with childhood onset’ including pyramidal signs, ataxia, posterior column sensory loss and mental retardation (Behr, 1909). While most of these cases apparently harboured heterozygous OPA1 mutations (Yu-Wai-Mann et al., 2010; Marelli et al., 2011; Pretegiani et al., 2011), the case presented by Schaaf et al. (2011) had the peculiar occurrence of compound heterozygosity for two different OPA1 mutations, the p.V903GfsX3 frameshift deletion and the p.I382M missense mutation, respectively, which suggested that bi-allelic OPA1 mutations may lead to a complicated form of optic atrophy, i.e. Behr syndrome. Most recently, Bonifert et al. (2014) further confirmed the occurrence of DOA ‘plus’ cases with bi-allelic OPA1 mutations, one of the alleles carrying the same p.I382M missense mutation.

We here add our observation of a similar bi-allelic OPA1 case with Behr syndrome. This proband is a 20-year-old Italian boy, born from unrelated parents (Fig. 1A, IV-4 in the pedigree), who presented a congenital nystagmus at birth and bilateral optic atrophy was recognized in the first year of life. He acquired autonomous walking at 14 months of age, but clumsy gait and frequent falls were reported. Motor difficulties and visual acuity worsened during childhood whereas cognitive development was normal. Brain MRI performed at the age of 4 and 7 years were normal. At 6 years of age, neurological examination showed ataxic-spastic gait with positive Romberg sign. Fundus exam confirmed bilateral optic atrophy (Fig. 1B). Tendon reflexes were brisk at the lower limbs and there was bilateral Babinski
sign. The patient also had bilateral talipes equinovarus. Nerve conduction studies showed delayed sensory-motor velocities with reduced amplitudes of motor potentials and loss or severe reduction of sensory action potentials. Somatosensory evoked potentials could not be recorded and motor evoked potentials showed absent potentials at lower limbs and delayed latency at upper limbs. Repeated urine organic acid gas chromatography/mass spectrometry analysis revealed mild elevation of 3-methylglutaconic acid (8–20 μmol/mmol creatinine). At muscle biopsy histology was normal and spectrophotometric assessment of the OXPHOS enzymatic activities was normal in muscle homogenate. Sural nerve biopsy demonstrated a marked reduction of the large-diameter myelinated axons (Fig. 1E–G). Teased fibre analysis disclosed regions of demyelination confined to the paranodal region, clustered in some axons, suggesting secondary demyelination. Ultrastructural examination confirmed the reduction of myelinated axons (not shown). A clinical reevaluation at 15 years of age demonstrated elevated serum lactic acid after standardized exercise (3.9 mM, normal range 0.6–2.4 mM) and optical coherence tomography revealed a generalized reduction of the retinal fibre layer thickness (Fig. 1D). At neurological examination, the patient was wheelchair bound due to severe sensory ataxia and spastic paraparesis and signs of cerebellar dysfunction, such as dysmetria, dysdiadochokinesia and positive Holmes rebound phenomenon, were evident. His vision was light perception with severe nystagmus in any direction of gaze. This clinical picture remained stable in the subsequent years.

Figure 1 (A) Reconstruction of the paternal and maternal genealogies of the proband with Behr syndrome (IV-4). The maternal side is remarkable for segregating isolated optic neuropathy in a dominant fashion associated with the heterozygous c.1705 + 1G > T splicing mutation. (B–D) Ophthalmological investigations. The fundus picture of the proband (IV-4) reveals severe optic atrophy with bilateral pale optic disc (B). The fundus picture of the mother (III-4) shows only a mild optic atrophy with temporal pallor of the optic discs bilaterally (C). Optical coherence tomography examination of retinal nerve fibre layer (RNFL) thickness in the proband shows a diffuse reduction of thickness compatible with the severe optic atrophy (D). (E–G) Semithin cross-section of the sural nerve biopsy. A marked depletion of large myelinated axons affects the sural nerve (E and F). At higher magnification some axons appear to have a thin myelin sheath related to the axonal diameter (G). (H–L) Brain MRI and 1HMR spectroscopy studies. On the left (H) the sagittal FSPGR (Fast SPoiled GRadient echo) T1 shows a mild cerebellar atrophy in the proband (IV-4), and on the right (I) a mild pathological accumulation of lactate (rectangle) was detected in the CSF of lateral ventriculi. On the axial FLAIR T2 sequence no signal changes were detected in the proband (IV-4) (J), whereas few focal hyperintensities, related to not specific areas of gliosis, in the cerebral white matter of the proband’s father (III-3) (K) and mother (III-4) (L) were present. TEMP = temporal; SUP = superior; NAS = nasal; INF = inferior; MRI = magnetic resonance imaging; HMR = proton magnetic resonance; CSF = cerebrospinal fluid; FLAIR = Fluid attenuated inversion recovery.
The 43-year-old proband’s mother (Fig. 1A, III-4 in the pedigree) had isolated optic atrophy, most evident on the temporal side (Fig. 1C), which segregated as a dominant trait in her family (Fig. 1A). The remaining neurological examination was normal. Nerve conduction velocities and somatosensory evoked potentials were normal. The other affected individuals in this family all suffered a non-syndromic optic atrophy, which started in childhood and progressively worsened, but remained as an isolated symptom.

The proband’s father and the elder sister (Fig. 1A, III-3 and IV-3, respectively) are healthy. In particular, neither signs of optic neuropathy nor abnormal nerve conduction velocities were recorded upon neuro-ophtalmological exam.

Both parents and the proband have been investigated by brain MRI and proton magnetic resonance spectroscopy (1H-MRS) in a 1.5 T scanner. There were no MRI structural or metabolic abnormalities in the father (III-3) and the mother (III-4) of the proband, except for a few focal hyperintensities in the cerebral white matter (Fig. 1K-L), whereas in the proband mild cerebellar atrophy (Fig. 1H,J) and pathological accumulation of lactate in the CSF of lateral ventriculi (Fig. 1I) were evident.

Direct sequencing of the entire coding regions of the OPA1 gene and the flanking intronic boundaries disclosed two heterozygous mutations. The first c.1705+1G>T mutation occurs in intron 17 and involves the highly conserved donor splice site consensus sequence. Analysis of cDNA revealed aberrant transcript splicing with the activation of a cryptic splice donor site in exon 17, which predicts a prematurely truncated gene product (data not shown). This haploinsufficiency mutation was also identified in the affected mother and in nine maternal relatives, three of which (III-10, III-14 and III-18) are clinically unaffected (Fig. 1A). The second mutation identified in the proband is missense, c.1146A>G (p.I382M), which lies in exon 12, being also present in the clinically unaffected father and sister. Interestingly this is the same missense mutation already reported in other bi-allelic cases (Schaaf et al., 2011; Bonifert et al., 2014). This mutation was found only in one other patient in Italy, out of 132 OPA1 complete sequences. This patient is currently under investigation, being another case of Behr syndrome with optic atrophy complicated by ataxia, but with only the heterozygous c.1146A>G (p.I382M) mutation at the moment (Leonardo Caporali and Valerio Carelli, unpublished data). In the Tübingen cohort of patients with bilateral optic atrophy (in most cases isolated optic atrophy) no further case with the c.1146A>G mutation was found among ~500 patients that underwent sequencing of all coding exons of the OPA1 gene. This mutation has also been found at low frequency (about 1/1000) in large public data sets (1000 Genome Project, NHLBI GO Exome Sequencing Project). Sequence analysis of the OPA3 gene excluded the presence of pathogenic mutations.

Fibroblasts were grown from skin biopsies obtained from the proband and both parents, after approval of the Internal Review Board and signed informed consent. In fibroblasts we first assessed mitochondrial network morphology, after loading mitochondria with Mitotracker Red and examination by fluorescence microscopy as previously reported (Zanna et al., 2008).

The mitochondrial network of fibroblasts grown in standard Dulbecco’s modified Eagle’s medium-glucose medium bearing the heterozygous c.1705+1G>T (III:4) and c.1146A>G (III:3) mutations, respectively was similar to controls (Fig. 2A-C), whereas the fibroblasts from the proband were scored into three categories, those with a typical normal filamentous network (Fig. 2D), those with a hyperfragmented pattern (Fig. 2E) and those with filamentous mitochondria containing balloon-like structures (Fig. 2F), each class being present at approximately the same percentage. To investigate whether these OPA1 mutations could affect the bioenergetic efficiency, we measured the rate of mitochondrial ATP synthesis in digitonin-permeabilized fibroblasts. Compared to controls, mitochondrial ATP synthesis driven by complex I substrates (pyruvate and malate) was increased in the proband’s father fibroblasts (III:3) bearing the c.1146A>G mutation, and incrementally reduced in the mother’s fibroblasts bearing the c.1705+1G>T (III:4) mutation and in the proband’s fibroblasts (IV:4) carrying both mutations. The latter was significantly lower compared to his father’s fibroblasts. A similar but less severe situation was observed for the ATP synthesis driven by the complex II substrate (succinate) (Fig. 2G). Overall, these results indicate that the biallelic mutation in the proband led to abnormal network dynamics and impaired ATP synthesis through complex I. Both parent’s mutations were not severe enough to impair mitochondrial network dynamics under the experimental conditions used, but the mother’s mutation impaired partially complex I-dependent ATP synthesis.

Ours and the other cases previously published (Yu-Wai-Mann et al., 2010; Marelli et al., 2011; Pretegiani et al., 2011; Schaaf et al., 2011; Bonifert et al., 2014), as well as further similar cases recently communicated by Bonneau and colleagues, all raise a few considerations. Our patient, who presented with a neurological syndrome characterized by congenital and severe optic atrophy, spastic paraplegia, peripheral neuropathy with axonal loss, cerebellar signs and mild 3-methylglutaconic aciduria, resembles both ‘Costeff’ and ‘Behr’ syndromes (Behr, 1909; Costeff et al., 1989). Thus, infantile severe syndromic optic neuropathies can be associated with both recessive OPA3 mutations and bi-allelic OPA1 mutations (Anikster et al., 2001; Bonifert et al., 2014 and this report). The biallelic OPA1 mutations found in our patient combine a classic OPA1 haploinsufficiency mutation, which can determine dominant isolated optic atrophy with reduced pene trance as in the mother’s genealogy, and a missense mutation that apparently was not able to lead to clinical symptoms per se in the father and the sister, but contributed consistently in modulating the phenotype in the compound heterozygote combination. In this regard, the p.I382M mutation might be considered as hypomorphic or with very low potential pathogenicity. A question can be raised if the other, apparently mono-allelic severe cases of ‘Behr’-like phenotype are truly such, or if a second mutation lies in the intronic regions or the promoter, as recently shown by Bonifert et al. (2014).

Our cell and magnetic resonance spectroscopy results strongly indicate a severe impairment of mitochondrial function in the proband, with hyperfragmented mitochondrial network morphology and deficient ATP synthesis driven by complex I substrates, which is reflected by the pathological lactate accumulation in the brain. Deeper investigations are needed on a pooled sample of these bi-allelic cases carrying the p.I382M change, to truly disentangle its real role in the pathogenesis of this syndrome, as well as...
Figure 2 (A–F) Mitochondrial network morphology. Control (A) and patient (B–F) fibroblasts were grown in Dulbecco’s modified Eagle’s medium-glucose and loaded with Mitotracker Red as described in Zanna et al. (2008). Representative out of eight similar images are shown for each individual. (G) ATP synthesis. Fibroblasts were treated with 50 μg/ml digitonin, the rate of ATP synthesis was measured as described in Zanna et al. (2008) and was normalized for citrate synthase (CS) activity. Data (mean ± SEM) were obtained from five controls and the three OPA1 mutated fibroblasts. The experiment was performed at least in triplicate. Asterisk denotes values significantly different (P < 0.05) by Kruskal-Wallis one-way ANOVA on Ranks. GPD = Glyceraldehyde 3-phosphate dehydrogenase.
other possible roles in predisposing to isolated optic neuropathy. The frequency of this allele in different populations should be also investigated.

In conclusion, the range of clinical phenotypes associated with OPA1 mutations, heterozygous or in biallelic combinations, further expands to Behr syndrome and possibly to other infantile disorders, as recently seen for MFN2 mutations (Renaldo et al., 2012). Fusion proteins, and more in general the machinery driving mitochondrial dynamics, are again highlighted for their crucial role in mitochondrial and cellular homeostasis, and it is anticipated that a wide range of human pathologies will be linked to genetic mutations affecting this pathway.

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