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SHORT COMMUNICATION

MRPL44 mutations cause a slowly progressive multisystem disease with childhood-onset hypertrophic cardiomyopathy

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Abstract Defects in mitochondrial translation may lead to combined respiratory chain deficiency and typically cause childhood-onset multisystem disease. Only recently, a homozygous missense mutation (c.467T>G, p.Leu156Arg) in *MRPL44*, encoding a protein of the large subunit of the mitochondrial ribosome, has been identified in two siblings with hypertrophic cardiomyopathy. Using exome sequencing, we identified two further unrelated patients harboring the previously reported mutation c.467T>G, p.Leu156Arg in *MRPL44* in the homozygous state and compound heterozygous with a novel missense mutation c.233G>A, p.Arg78Gln, respectively. Both patients presented with childhood-onset hypertrophic cardiomyopathy, which seems to be the core clinical feature associated with MRPL44 deficiency. However, we observed several additional clinical signs and symptoms including pigmentary retinopathy, hemiplegic migraine, Leigh-like lesions on brain MRI, renal insufficiency, and hepatopathy. Our findings expand the clinical spectrum associated with *MRPL44* mutations and indicate that *MRPL44*-associated mitochondrial dysfunction can also manifest as a progressive multisystem disease with central nervous system involvement. Of note, neurological and neuro-ophthalmological impairment seems to be a disease feature of the second and third decades of life, which should be taken into account in patient management and counseling.

Keywords Oxidative phosphorylation · OXPHOS · Mitochondrial ribosome · Mitochondrial disease

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Introduction

The mitochondrial ribosomal proteins are encoded by nuclear genes and imported into mitochondria where they assemble together with mitochondrial ribosomal RNAs into ribosomes. This mitochondrial translation machinery is responsible for synthesizing 13 structural components of the mitochondrial respiratory chain. Defects in mitochondrial ribosomal proteins are an emerging cause of childhood-onset disease and may present with variable clinical phenotypes [1–4].

In 2013, Carroll et al. identified two siblings with a homozygous missense mutation in *MRPL44*, which encodes a structural component of the mitochondrial large ribosomal subunit [5]. Both children presented with infantile cardiomyopathy, leading to a fatal infection-triggered cardiac decompensation in one child and a rather mild, slowly progressive clinical course in the other child.

Here, we describe two unrelated patients with compound heterozygous and homozygous *MRPL44* missense mutations, respectively, and hypertrophic cardiomyopathy as a unifying clinical feature. A long-term clinical course was characterized by a rather slowly progressive multisystem disease, involving skeletal muscle, liver, kidneys, and central nervous system.

Materials and methods

Exome sequencing

We performed exome sequencing as described previously to investigate the molecular basis underlying the mitochondrial disease presentation in these two patients [6]. In brief, coding DNA fragments were enriched with a SureSelect Human All Exon 50Mb V5 Kit (Agilent, Santa Clara, CA, USA), and sequencing was performed on a HiSeq2500 system (Illumina, San Diego, CA, USA). Reads were aligned to the human genome assembly hg19 (UCSC Genome Browser) with Burrows-Wheeler Aligner (BWA, v.0.5.87.5), and detection of genetic variation was performed using SAMtools (v 0.1.18), PINDEL (v 0.2.4t), and ExomeDepth (v1.0.0). More than 90 % (91 and 97 %) of the targeted sequences were covered at least 20-fold.

Results

Case report patient 1

This 8-year-old girl is the first child of healthy non-consanguineous German parents. She was born at term after a normal pregnancy. Transitory feeding difficulties were noted at 6 months of age; however, no cause was found. Cognitive and motor development in the first 2 years of life was normal. However, the girl was less active than other children of her age and quickly exhausted after physical exercise. An abnormal fat distribution pattern with unusual buccal fat pads was recorded.

At the age of 3 years, a severe non-obstructive hypertrophic cardiomyopathy was diagnosed. A metabolic screening and search for cardiotropic viruses were negative. Serum lactate was moderately increased (up to 5 mmol/L; normal range <2.2), particularly after prolonged fasting. Free carnitine levels were mildly reduced, and treatment with L-carnitine 70 mg/kg/day (and propranolol) led to an improvement in physical capacity. Electron microscopic evaluation of endomyocardial biopsies demonstrated mitochondrial proliferation and elongation, which pointed to a potential energy metabolism disorder. Therefore, a skin biopsy was performed. Biochemical analysis of the oxidative phosphorylation system (OXPHOS) in fibroblasts revealed an isolated complex IV deficiency (345 mU/U citrate synthase; control range 680–1190 mU/U citrate synthase).

The further clinical course was characterized by mild hepatopathy with transiently elevated liver transaminases (AST up to 100 U/l; norm <48; GGT up to 87 U/l; norm <22), moderate exercise intolerance, and muscle weakness. Cognitive development remained normal; at the current age of 8 years, she attends elementary school and has no learning difficulties. At last follow-up, cardiac function remains stable (moderate non-obstructive hypertrophic cardiomyopathy; ejection fraction of 35 %).

Case report patient 2

This 26-year-old female patient is the third child of healthy non-consanguineous German parents with two healthy daughters. She was born at term after a normal pregnancy. On the second day of life, she developed severe metabolic acidosis (pH 7.17, BE −21 mmol/L), treated in intensive care setting. Since no underlying infection was identified, a metabolic disorder was suspected, but no cause was found at that time.

Motor development milestones were normal. Learning difficulties, short-term memory, and concentration problems were noted from the age of 3 years. Exercise-induced muscle pain was also present from the age of 3.

From the age of 14 years, she developed frequent episodes of hemiplegic migraine, with visual aura followed by headache and transient flaccid hemiplegia and facial paresis, hemiparesthesias, slurred speech, and dysphasia, up to five episodes per week. Residual neurological deficits, including word-finding difficulties, paresthesias, and difficulty writing, were noted after a severe episode aged 16.

At that time, tapetoretinal dystrophy was diagnosed, with pigment deposits in the retina, narrowing of retinal arterioles, and visual field defects, which progressed at last follow-up to bilateral ring-shaped scotomas. At the same time, hypertrophic non-obstructive cardiomyopathy was diagnosed. An

endomyocardial biopsy showed hypertrophy of the heart muscle, subendocardial myocytolysis, and perivascular and interstitial fibrosis. Biochemical analysis of the respiratory chain enzymes in heart tissue revealed severe complex IV deficiency (211 mU/U citrate synthase; normal range 500–1100 mU/U citrate synthase) and moderate complex I deficiency (5.1 mU/mg; normal range 5.5–52 mU/mg).

Brain MRI at 25 years showed symmetrical T_2-weighted hyperintensities in the thalamus, basal ganglia, and cerebellum (Fig. 1). MR spectroscopy revealed a discrete lactate peak.

At last follow-up, aged 26, the neurological status was relatively stable. Two episodes of cardiac insufficiency with pulmonary edema led to emergency admission to the intensive care unit, which were controlled by intravenous diuretics. Currently, she has two mild attacks of hemiplegic migraine per year, exercise-induced muscle pain, dyspnea on exertion, learning and concentration difficulties, and impaired fine motor skills. In addition, she developed renal insufficiency (GFR 46 ml/min; normal ≥60). At last follow-up, cardiac function was stable (left ventricular ejection fraction of 30 %, global hypokinetic left ventricle).

Genetic studies

A search for genes carrying rare homozygous or predictively compound heterozygous variants (MAF in 4,500 control exomes <0.1 %) identified 8 and 17 genes, respectively. In both analyses, *MRPL44* was the only gene which had previously been linked to mitochondrial dysfunction [5, 7]. In patient 1, we identified the previously reported missense mutation c.467T>G, p.Leu156Arg and a novel missense mutation, c.233G>A, p.Arg78Gln. Visualization of mate pair reads confirmed a compound heterozygous state of the mutations (Supplementary Fig. 1). In patient 2, we identified the mutation c.467T>G, p.Leu156Arg in the homozygous state with both parents being heterozygous carriers as shown by confirmatory Sanger sequencing. Both variants are very rare in public databases (eight heterozygous carriers of the change c.467T>G

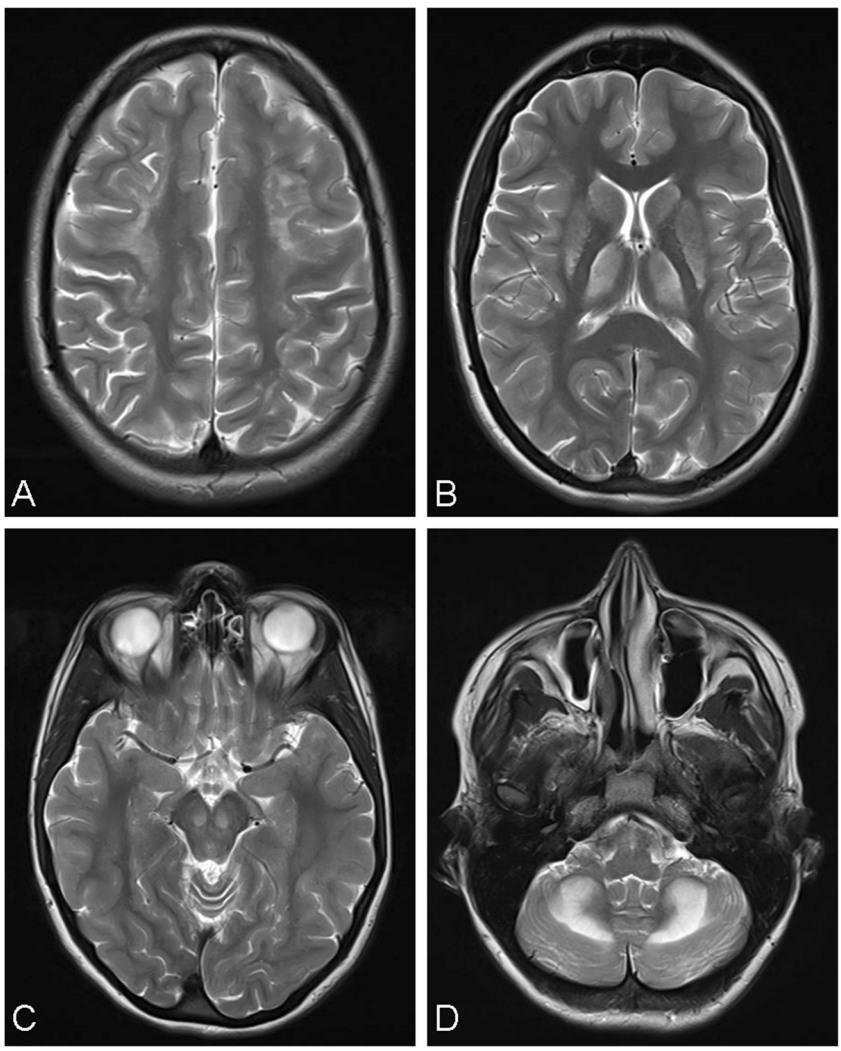

Fig. 1 Brain MRI of patient 2 (T_2-weighted, axial view) at 25 years of age showing diffuse subcortical white matter abnormalities (**a**), symmetrical hyperintensities in the basal ganglia (**b**) and midbrain (**c**), as well as extensive symmetrical signal abnormalities in the cerebellum (**d**). MR spectroscopy (not shown) revealed a discrete lactate peak

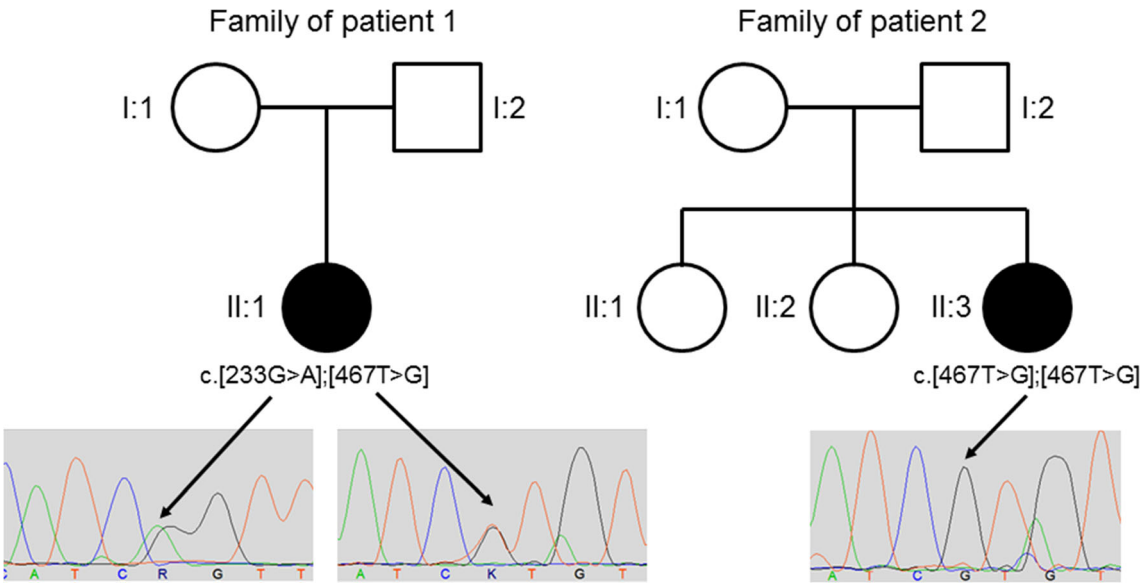

Fig. 2 Pedigrees of investigated families and electropherograms showing identified *MRPL44* mutations. Mutation status of affected (*closed symbols*) and unaffected (*open symbols*) family members is depicted

and one with c.233G>A in 122,890 control alleles; Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: http://exac.broadinstitute.org) [11/2014]). Both mutations change highly conserved amino acids and are accordingly predicted to have a detrimental impact on protein function by several software tools (PolyPhen 2, Sift). For the c.467T>G, p.Leu156Arg mutation, it has been shown that it results in severely reduced MRPL44 levels [5]. Pedigrees of both investigated families and electropherograms are depicted in Fig. 2.

Discussion

MRPL44-associated mitochondriopathy was recently described in two Finnish siblings, aged 6 months and 14 years, with hypertrophic cardiomyopathy as a leading symptom and combined respiratory complex I and IV deficiency in heart tissue and isolated complex IV deficiency in fibroblasts [5]. Based on their findings, the authors speculated that the defect might generate a tissue-specific manifestation.

The patients described here support the idea that heart muscle is specifically vulnerable to the metabolic defect. However, our report expands the clinical spectrum of *MRPL44*-associated mitochondrial disease and indicates that the defect also causes a slowly progressive multisystem disease, especially during the later clinical course. This is exemplified in patient 2, who developed myopathy, hemiplegic migraine, pigmentary retinopathy, renal insufficiency, and a Leigh-like lesion pattern on brain MRI (see Fig. 1). Moreover, also one of the patients reported by Carroll et al. developed ophthalmological abnormalities with granular pigmentation in the retina (which might be a pre-stage of what we see in patient 2) and was diagnosed with transiently elevated liver transaminases as a sign of mild hepatopathy (comparable to patient 1) [5].

The functional studies of Carroll et al. demonstrated that severely decreased MRPL44 results in an assembly defect of the large ribosomal subunit but only mildly affected mitochondrial translation. Tissue-specific thresholds and different compensatory mechanisms may contribute to the slow disease progression in the majority of affected patients. The specific vulnerability of mitochondrial complex IV, also found in *MRPL12* mutations, may be caused by a specific interaction with complex IV chaperones or assembly factors [4, 5]. On the other hand, Ruzzenente et al. showed that newly synthesized COXI subunits have a high turnover, which may result in an increased sensitivity of complex IV to decreased synthesis of its subunits [8].

In conclusion, based on the clinical data available, *MRPL44* mutations should be considered in patients with slowly progressive mitochondrial multisystem disease, especially in the presence of cardiomyopathy. Of note, neurological and neuro-ophthalmological clinical features seem to occur during the second and third decades of life, which should be taken into account in patient management and genetic counseling. In general, further clinical descriptions will be required to evaluate the specific clinical spectrum of patients with *MRPL44* mutations.

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