Original research

Recessive MECR pathogenic variants cause an LHON-like optic neuropathy

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ABSTRACT

Background  Leber’s hereditary optic neuropathy (LHON) is a mitochondrial disorder characterised by complex I defect leading to sudden degeneration of retinal ganglion cells. Although typically associated with pathogenic variants in mitochondrial DNA, LHON was recently described in patients carrying biallelic variants in nuclear genes DNAJC30, NDUFS2 and MCAT. MCAT is part of mitochondrial fatty acid synthesis (mtFAS), as also MECR, the mitochondrial trans-2-enoyl-CoA reductase. MECR mutations lead to a recessive childhood-onset syndromic disorder with dystonia, optic atrophy and basal ganglia abnormalities.

Methods  We studied through whole exome sequencing two sisters affected by sudden and painless visual loss at young age, with partial recovery and persistent central scotoma. We modelled the candidate variant in yeast and studied mitochondrial dysfunction in yeast and fibroblasts. We tested protein lipoylation and cell response to oxidative stress in yeast.

Results  Both sisters carried a homozygous pathogenic variant in MECR (p.Arg258Trp). In yeast, the MECR R258W mutant showed an impaired oxidative growth, 30% reduction in oxygen consumption rate and 80% decrease in protein levels, pointing to structure destabilisation. Fibroblasts confirmed the reduced amount of MECR protein, but failed to reproduce the OXPHOS defect. Respiratory complexes assembly was normal. Finally, the yeast mutant lacked lipoylation of key metabolic enzymes and was more sensitive to H2O2 treatment. Lipoic acid supplementation partially rescued the growth defect.

Conclusion  We report the first family with homozygous MECR variant causing an LHON-like optic neuropathy, which pairs the recent MCAT findings, reinforcing the impairment of mtFAS as novel pathogenic mechanism in LHON.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Leber’s hereditary optic neuropathy (LHON) is a rare disease due to genetic defects affecting mitochondrial complex I function, most typically involving the mitochondrial genome but rarely also autosomal recessive. Recently, a single case was reported with MCAT variants and LHON-like phenotype, linking the mitochondrial fatty acid synthesis (mtFAS) pathways to LHON pathogenesis.

WHAT THIS STUDY ADDS

⇒ This study provides evidence that MECR is a new gene for autosomal recessive LHON, further supporting mtFAS as a novel pathway relevant for LHON and lipoic acid may be beneficial.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Further investigations are necessary to well characterise the novel link between mtFAS and LHON. However, MCAT should be added to the molecular diagnostic pathway for LHON.

BACKGROUND

Leber hereditary optic neuropathy (LHON (MIM: 353000)) is a mitochondrial optic neuropathy characterised by sudden degeneration of retinal ganglion cells leading to subacute bilateral loss of central vision, with limited occurrence of spontaneous recovery. The disease typically shows incomplete penetration, male prevalence and may be triggered by tobacco smoking.

LHON is the most common disease caused by mtDNA pathogenic variants, with a minimum prevalence reaching 3.22 per 100 000. Three variants in respiratory complex I subunits are found in about 90% of pedigrees, m.3460G>A/MT-ND1, m.11778G>A/MT-ND4 and m.14484T>C/MT-ND6, while several rare mtDNA point mutations or combinations of rare polymorphisms account for some of the remaining 10%.

Recently, an LHON-like phenotype with autosomal recessive inheritance has been associated with pathogenic variants in nuclear genes. The most relevant is DNAJC30, encoding a newly characterised mitochondrial chaperone that promotes complex I turnover. Several DNAJC30 pedigrees with a few pathogenic variants are described, all presenting with a mostly mild LHON phenotype (LHONA, MIM #619382), except for a few cases with Leigh syndrome. A single case of isolated recessive LHON was also reported with compound heterozygous variants in NDUFS2 gene, while
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biallelic variants in this core subunit of complex I are usually associated with syndromic CI deficiency (MCIDN, MIM #618228). Biallelic variants in another subunit of complex I, NDUFAL2, were also recently associated with a phenotypic spectrum ranging from movement disorders (dystonia and/or spasticity) to isolated optic atrophy, besides a more typical presentation of Leigh syndrome. An evocative acute/subacute vision loss was reported, however, the ophthalmological description of these NDUFAL2 optic atrophy cases was not detailed enough to draw firm conclusions about similarities with LHON. Finally, an LHON-like phenotype has been reported in one pedigree with pathogenic variants in MCAT, encoding the mitochondrial malonyl-CoA-acyl carrier protein transacylase. This is a key enzyme in mitochondrial fatty acid synthesis (mtFAS), which transfers malonyl-CoA to the mitochondrial acyl carrier protein NDUFAB1. Interestingly, the previous cases with homozygous variants in MCAT also presented with recessive optic atrophy, but with a progressive course, not obeying the LHON hallmark of subacute onset of visual loss.

The MECR gene encodes the mitochondrial trans-2-enoyl-CoA reductase enzyme, also involved in mtFAS. Among the fatty acids synthesised by mtFAS, there is octanoic acid, the precursor of lipoic acid (LA), a cofactor involved in the activity of several mitochondrial enzymes, among which the pyruvate dehydrogenase, the α-ketoglutarate dehydrogenase, the branched-chain keto acid dehydrogenase, the 2-oxoadipate dehydrogenase and the glycine cleavage system.

Pathogenic variants in MECR are associated with a recessive childhood-onset disorder characterised by dystonia, optic atrophy and basal ganglia abnormalities (DYTOAB, MIM: #617282), also described with the acronym MEPAN (mitochondrial enoyl coA reductase protein-associated neurodegeneration). A total of eight families have been described with MECR-associated disease, all with a fairly uniform clinical presentation.

We here describe the first family in which two siblings with homozygous MECR variants show an isolated optic neuropathy phenotype, with subacute onset. Thus, we propose MECR as another gene related to rare forms of LHON-like autosomal recessive optic neuropathy.

METHODS

Clinical evaluation

The study adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from patients. Clinical assessment included best-corrected visual acuity by Snellen’s chart, colour vision tests (Ishihara test), slit-lamp biomicroscopy, Goldmann applanation tonometry, colour fundus photography, optical coherence tomography (OCT; Cirrus, Carl Zeiss Meditec, Dublin, California, USA; DRI Triton, Topcon, Tokyo, Japan), automated visual field test (Humphrey Field Analyzer, protocol Sita Standard 30-2; Zeiss, San Leandro, California). OCT protocols included the evaluation of peripapillary retinal nerve fibre layer (RNFL) thickness (3.4 acquisition protocol) and segmentation analysis of the macula (ganglion cell layer (GCL) between RNFL and the inner nuclear layer boundaries).

Genetic study

Whole genome sequencing (WES) was performed on DNA from patient 1 extracted from peripheral blood. The sample library was prepared and enriched using TruSeq Rapid Exome Kit (Illumina), then sequenced with 150bp paired-end reads on a NextSeq 500 instrument (Illumina). Bioinformatic analysis followed the GATK Best Practices workflow for germline variant discovery, aligning to reference genome GRCh37/hg19. We prioritised rare variants compatible with autosomal recessive inheritance in genes encoding mitochondrial proteins. Variants of interest were classified according to the America College of Human Genetics (ACMG) guidelines. Next Generation Sequencing (NGS) findings were confirmed by Sanger sequencing on a 3500 Dx Genetic Analyzer (Applied Biosystems). H3M2 tool was used for the identification of runs of homozygosity (ROHs) from WES alignments. We also checked the proband’s WES for pathogenic CNVs by combining four tools: ExomeDepth, CoNIFER, XHMM, cn.MOPS.

Fibroblast model

Fibroblast cell lines derived from both patients were generated. The full description of cell lines and culture conditions, mitochondrial respiration evaluation, Western blot, antibodies and respiratory supercomplexes assembly by BN-Page and CI-in gel activity (CI-IGA) is available in online supplemental material.

Yeast model

The variant found in patients was studied in a yeast model disrupted in ETR1, the orthologous gene of MECR, and expressing human MECR. A detailed description of yeast model methods used for phenotypic analysis is available in the online supplemental material.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance followed by a post hoc Bonferroni test for multiple comparisons or by t-test.

RESULTS

Patient 1: case report

The proband is an early 50s woman who was first noticed for low vision at 6 years, detected during routine eye examinations at school. The past clinical charts showed at 22 years a reduction of visual acuity, 0.4 in her right eye (RE) and 0.5 in her left eye (LE), associated with a small central scotoma at visual fields.

Moreover, the patient reported two episodes of sudden and painless visual loss in RE at 28 and 44 years followed by recovery of visual function after 1 month. Visual fields at 29 years demonstrated a bilateral central scotoma (MD −4.29 in RE and MD −4.36 in LE) (online supplemental figure 1A).

Her medical history was remarkable for headache since the age of 12 years and mild sensorineural hearing impairment since she was 45 years. At 40 years of age, she reported cardiac arrhythmic problems (paroxysmal tachycardia) for which she is taking beta-blockers.

Patient 1: ophthalmological findings

We observed the patient 1 month after visual loss at 44 years and subsequent recovery of visual acuity. At ophthalmological evaluation, she had visual acuity of 0.32 in RE and 0.4 in LE. Defective colour vision was evident in both eyes (Ishihara test RE 6/12 and LE 8/12). Visual fields showed bilateral central scotoma (MD −6.09 in RE and MD −5.84 in LE) (online supplemental figure 1A).

At fundus examination, a small optic disc with central small excavation and temporal pallor was observed. OCT showed a diffuse reduction of the RNFL (average thickness RE 79 μm and LE 73 μm) and GCL (average thickness OU 60 μm), associated with a small optic disc area (OD 1.57 mm² and LE 1.50 mm²) (online supplemental figure 1C).
At the last examination, after a 9-year follow-up, the patient was stable, without significant changes in visual acuity and colour vision. Similarly, a stable central scotoma was noticed at visual fields (MD −3.18 in RE and MD −3.45 in LE) (figure 1A) and unchanged RNFL and GCL thinning at OCT were evident (figure 1A).

**Patient 2: case report**

This patient is the proband’s sister, now in the early 40s. She referred a sudden and painless visual loss in both eyes at 18 years during physical activity followed by slowly progressive recovery of vision. The past clinical charts demonstrated visual loss up to 0.05 in both eyes, associated with small central scotoma at visual fields. At 19 years, she had a bilateral central scotoma (MD −4.35 in RE and MD −3.39 in LE) (online supplemental figure 2A).

Her medical history was remarkable for headache since the age of 14 years and mild sensorineural hearing impairment since 38 years.

**Patient 2: ophthalmological findings**

The ophthalmological evaluation at 34 years of age disclosed visual acuity of 1.0 in RE and 0.8 in LE. Colour vision defect was evident in both eyes (Ishihara test RE 3/12 and LE 5/12). Visual fields showed a central scotoma (MD −3.39 in RE and MD −3.38 in LE) (online supplemental figure 2B). Ophthalmoscopy revealed a small optic disc with central small excavation and temporal pallor (online supplemental figure 2C). OCT showed a diffuse reduction of the RNFL associated with a small optic disc area (online supplemental figure 2C).

At the last examination, after a 7-year follow-up, the patient reported no further deterioration of visual function, the visual acuity and colour Ishihara test were unchanged. Visual fields revealed a stable central scotoma (MD −5.16 in RE and MD −3.51 in LE) (figure 1B). OCT did not show any change of RNFL and GCL thickness (figure 1B).

**Genetic analysis**

We performed WES on the proband (II-1, figure 1C) with a mean coverage of 79X, and 96% of target bases were above 20X. We detected a homozygous variant in the *MECR* gene (NM_016011.5), segregating also in the affected sister (II-1, figure 1C), while no likely pathogenic CNV was detected. The identified c.772C>T variant leads to the missense change p.Arg258Trp and is ultra-rare in GnomAD population database V2.1.1, with max MAF 6.36X. This variant has been previously reported in two families with MECR dysfunction and typical DYTOABG/MEPAN phenotype, but in compound heterozygosity with loss-of-function variants, and accordingly to all reported evidence is classified as pathogenic according to ACMG guidelines.

In this family, the p.Arg258Trp was found inside a large ROH of 3.16 Mb, pointing to a possible parental relatedness. The main clinical and genetic findings of published cases with the additional family presented here are summarised in table 1.

**Yeast model**

The Arg258 residue is conserved in fungi and animals (figure 2A), and we confirmed that human *MECR* wild-type allele (*MECR*<sub>wt</sub>) can partially complement the oxidative growth of the etr1Δ *S. cerevisiae* strain, as already reported (figure 2B). When the strain was transformed with *MECR* harbouring the Arg258Trp variant (*MECR*<sub>R258W</sub>), the mutant showed a reduced oxidative
### Table 1  MECR patients cohort

<table>
<thead>
<tr>
<th>Affected individual (ref)</th>
<th>Origin</th>
<th>Nucleotide and protein variants</th>
<th>Age at onset of dystonia</th>
<th>Age at onset of optic atrophy</th>
<th>Intellect</th>
<th>MRI involvement</th>
<th>Age at last visit</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Family A, II:1)</td>
<td>Ashkenazi Jewish</td>
<td>c.(695G&gt;A);(855T&gt;G) p.((Gly232Glu));(Tyr285Ter)</td>
<td>Early childhood</td>
<td>Mid-childhood</td>
<td>Preserved</td>
<td>Bilateral hyperintense T2 signal in the putamen</td>
<td>Late 40s</td>
<td>–</td>
</tr>
<tr>
<td>2 (Family B, II:2)</td>
<td>Mixed Jewish</td>
<td>c.(695G&gt;A);(830+2dup) p.((Gly232Glu));(?)</td>
<td>1–5 y</td>
<td>NA</td>
<td>Preserved</td>
<td>Bilateral hyperintense pallidal T2 signal</td>
<td>&lt;5 y</td>
<td>–</td>
</tr>
<tr>
<td>3 (Family C, II:2)</td>
<td>Ashkenazi Jewish</td>
<td>c.(695G&gt;A);(830+2dup) p.((Gly232Glu));(?)</td>
<td>1–5 y</td>
<td>5–10 y</td>
<td>Preserved</td>
<td>Bilateral hyperintense T2 signal in the dorsal striatum</td>
<td>40s</td>
<td>–</td>
</tr>
<tr>
<td>4 (Family C, II:8)</td>
<td>Ashkenazi Jewish</td>
<td>c.(695G&gt;A);(830+2dup) p.((Gly232Glu));(?)</td>
<td>1–5 y</td>
<td>10–15 y</td>
<td>Preserved</td>
<td>NA</td>
<td>Late 20s</td>
<td>–</td>
</tr>
<tr>
<td>5 (Family D, II:1)</td>
<td>Tunisian</td>
<td>c.(854A&gt;G);(854A&gt;G) p.((Tyr285Cys));((Tyr285Cys))</td>
<td>5–10 y</td>
<td>5–10 y</td>
<td>Relatively preserved (mild concentration difficulties)</td>
<td>Bilateral hyperintense T2 signal in the dorsal putamen</td>
<td>&lt;10 y</td>
<td>–</td>
</tr>
<tr>
<td>6 (Family E, II:1)</td>
<td>Anglo-Saxon</td>
<td>c.(772C&gt;T);(247_250del) p.((Arg258Trp));((Asn83HisfsTer4))</td>
<td>1–5 y</td>
<td>5–10 y</td>
<td>Deterioration of linguistic skills and executive functions to extremely low range at 9 y</td>
<td>Bilateral hyperintense pallidal T2 signal with cavitation and lactate peak on MRS</td>
<td>Late 10s</td>
<td>Choreoathetosis, retinopathy, cognitive decline (trans-2-enoyl-CoA reductase deficiency)</td>
</tr>
<tr>
<td>7 (Family E, II:3)</td>
<td>Anglo-Saxon</td>
<td>c.(772C&gt;T);(247_250del) p.((Arg258Trp));((Asn83HisfsTer4))</td>
<td>1–5 y</td>
<td>1–5 y</td>
<td>Low average verbal comprehension with extremely low function on the other WISC IV indices</td>
<td>Bilateral hyperintense pallidal T2 signal with lactate peak on MRS</td>
<td>10s</td>
<td>Choreoathetosis, retinopathy, cognitive decline (trans-2-enoyl-CoA reductase deficiency)</td>
</tr>
<tr>
<td>8 (Family F, II:2)</td>
<td>Turkish</td>
<td>c.(772C&gt;T);(1009C&gt;T) p.((Arg258Trp));((Arg337Ter))</td>
<td>1–5 y</td>
<td>1–5 y</td>
<td>NA</td>
<td>Hyperintense signal and cystic changes in the putamen, caudate nucleus and globus pallidus in addition to generalised mild cerebral atrophy</td>
<td>&lt;5 y</td>
<td>–</td>
</tr>
<tr>
<td>9 (Family G, II:1)</td>
<td>–</td>
<td>c.(830+2dup);(−39G&gt;C) p.?(?)</td>
<td>&lt;1 y</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>NA</td>
<td>Delayed motor milestones and hypotonia, which evolved to include spasticity, an atactic gait and progressive loss of motor skills</td>
</tr>
<tr>
<td>10 (Family G, II:2)</td>
<td>–</td>
<td>c.(830+2dup);(−39G&gt;C) p.?(?)</td>
<td>&lt;1 y</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>NA</td>
<td>Delayed motor milestones and hypotonia, which evolved to include spasticity, an atactic gait and progressive loss of motor skills</td>
</tr>
<tr>
<td>11 (Family H, II:1)</td>
<td>Chinese</td>
<td>c.(910G&gt;T);(910G&gt;T) p.((Asp304Tyr));((Asp304Tyr))</td>
<td>5–10 y</td>
<td>NA</td>
<td>Preserved</td>
<td>Bilateral hyperintense globus pallidus and cerebral peduncle T2 signals, with globus pallidus liquefaction and necrosis</td>
<td>10s</td>
<td>–</td>
</tr>
<tr>
<td>12 (Family I, II:1) (This study)</td>
<td>Italian</td>
<td>c.(772C&gt;T);(772C&gt;T) p.((Arg258Trp));((Arg258Trp))</td>
<td>–</td>
<td>5–10 y</td>
<td>Preserved</td>
<td>–</td>
<td>50s</td>
<td>Optic atrophy and mild sensorineural impairment</td>
</tr>
<tr>
<td>13 (Family I, II:2) (This study)</td>
<td>Italian</td>
<td>c.(772C&gt;T);(772C&gt;T) p.((Arg258Trp));((Arg258Trp))</td>
<td>–</td>
<td>15–20 y</td>
<td>Preserved</td>
<td>–</td>
<td>30s</td>
<td>Optic atrophy and mild sensorineural impairment</td>
</tr>
</tbody>
</table>

MECR coding DNA reference sequence NM_016011.5, MECR protein reference sequence NP_057095.4. MRS, magnetic resonance spectroscopy; NA, not available; y, years.
growth compared with the strain harbouring MECR<sup>wt</sup> on a medium supplemented with 2% ethanol, indicating the variant is associated with an oxidative phenotype (figure 2B). This result was confirmed by the oxygen consumption rate (OCR), which was similar to that of the etr1Δ strain transformed with the empty plasmid and decreased by 30% compared with the strain transformed with MECR<sup>wt</sup> (figure 2C). By measuring the steady-state levels of MECR protein, we observed two bands, whose size is compatible with the unprocessed MECR protein and with the functional MECR protein in which the mitochondrial localisation signal was removed: in the MECR<sup>R258W</sup> strain, the MECR levels were decreased by 80%, suggesting that reduced levels of mutant MECR contributed to the phenotypic defects (figure 2D).

**Fibroblasts analysis**

To gain further insights into the consequences of the MECR<sup>R258W</sup> mutation, we investigated fibroblasts derived from the two patients carrying homozygous MECR variant here reported. Western blot analysis on total cellular lysates indicated that MECR protein levels were significantly decreased in mutants compared with controls. As observed in the yeast model, these results confirm that MECR<sup>R258W</sup> mutation causes protein reduction (figure 3A,B).

Since the MECR<sup>R258W</sup> mutation in yeast showed an oxidative phosphorylation (OXPHOS) defect, we investigated the OCR after having incubated fibroblasts in a galactose-containing medium for 48 hours, thus forcing cells to rely on OXPHOS...
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Figure 3  Characterisation of patient-derived fibroblasts carrying MECR<sup>R258W</sup> variant. (A) Western blot of MECR; GAPDH was used as loading control. A representative blot of three independent experiments (biological replicates) is shown. (B) Densitometry of MECR content. All data are means and SD Statistical analysis was performed by t-test. **: p<0.01. (C) OCR expressed as picomoles O2/min, normalised for protein content, under basal conditions and after injection of oligomycin (O), carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP; F), rotenone (R) and antimycin A (A). Data are expressed as means ± SD of three independent experiments (biological replicates). (D) Basal, ATP-linked, maximal and CI respiration. All values are means and SD of three independent experiments (biological replicates). (E) Western blot of OXPHOS subunits; GAPDH was used as loading control. A representative blot of three independent experiments is shown for each protein. (F) Densitometry of OXPHOS subunits of three independent experiments (biological replicates). All data are means and SD. (G) CI-IGA of SCs from mitoplasts obtained from control and mutant cells. One representative experiment of three is shown. IGA, in gel activity.

Rescue of the phenotype by LA in yeast

It was previously reported that deletion of ETR1 is associated with undetectable levels of lipoylated Lat1 and Kgd2, the dihydrolipoamide acetyltransferase subunit and the dihydrolipoyl trans-succinylase subunit, respectively, of the pyruvate dehydrogenase and of the α-ketoglutarate dehydrogenase, due to the lack of LA biosynthesis.13 22 We observed lipoylated Lat1 and Kgd2 in the MECR<sup>wt</sup> strain, whereas the two proteins are undetectable in the MECR<sup>R258W</sup> strain (figure 4A), suggesting that the variant deeply affected the ability of the protein to synthesise the LA precursor. LA is also a potent ROS scavenger, and it was previously demonstrated that a decrease in its level due to variants in genes involved in its synthesis is associated with increased sensitivity to oxidant agents such as H2O2.23 By measuring the sensitivity of the MECR<sup>wt</sup> and MECR<sup>R258W</sup> strain to H2O2, we observed that the presence of the variant is associated with a decreased growth (figure 4B) and a decreased viability (figure 4C).

The yeast model allowed evaluation of whether the supplementation with LA could ameliorate the detrimental phenotype.
induced by the variant. The growth rate of the $\text{MECRI}^{\text{wt}}$ strain in liquid YP medium supplemented with ethanol was lower compared with the $\text{MECRI}^{\text{wt}}$ strain. When the $\text{MECRI}^{\text{R258W}}$ strain was supplemented with LA at a high concentration (40 µg/mL), a slight but significant increase in the growth rate was observed after 72 and 96 hours (figure 4D). By measuring the levels of lipoylated Lat1 or Kgd2 in the mutant strain, no proteins were detectable even at a high concentration of LA (figure 4E), indicating that the rescue of the growth was not due to lipoylation of such proteins.

To evaluate whether the improved oxidative growth was due to the intrinsic antioxidant characteristics of LA, we measured the effect of pretreatment with LA at different concentrations after exposure of the mutant strain to 1 mM $\text{H}_2\text{O}_2$ for 2 hours. Pretreatment with at least 4 µg/mL LA increased the viability by approximately 20% (figure 4F):
this value was slightly lower compared with that of MECR\textsuperscript{27} strain exposed to H\textsubscript{2}O\textsubscript{2} without LA pretreatment. Thus, we confirm the beneficial effects of LA supplementation to reduce oxidative stress.

DISCUSSION

We described two sisters presenting with optic atrophy: the proband apparently had a childhood onset, with subsequent subacute episodes, whereas the younger sister showed a subacute onset resembling LHON. The co-existence of childhood onset and subacute loss of central vision at 18 years of age in the same family summarises the known spectrum of LHON.\textsuperscript{1} Nonetheless, the multiple subacute episodes reported in the proband with childhood onset are a remarkable feature, not typically seen in LHON. The only extracocular feature was a mild sensorineural hearing impairment, not standard in LHON, even though the involvement of the acoustic nerve has been reported also in LHON.\textsuperscript{26}

The genetic investigations identified a known recessive pathogenic MECR variant, previously found in compound heterozygosity with null alleles and associated with the phenotype of DYT1OBG/MEPAN. This same variant in homozygous state was here associated with the novel phenotype of LHON-like isolated optic neuropathy and mild sensorineural hearing impairment. The p.Arg258Trp variant strongly decreased the MECR protein levels, both in patients' fibroblasts and in yeast, suggesting that instability of the mutant protein, rather than a decrease in its activity, is the main cause of the pathological phenotype. Despite a childhood onset of the disease in one case, like the syndromic published cases, the two affected siblings in our family did not show any sign of dystonia or MRI abnormalities, even after 40 years of age. It is possible that the residual quantity of MECR protein observed in mutant fibroblasts is sufficient for sustaining some biochemical activity, thus explaining a less severe phenotype compared with the described paediatric cases.

The damaging effect of p.Arg258Trp variant was confirmed through yeast modelling, where the reduction of MECR protein levels is paired with no detectable lipoylation in mitochondrial metabolic enzymes such as Lac1 or Kgd2. This defect translates into impaired oxidative growth and OCR, as well as reduced protection from oxidative stress induced by H\textsubscript{2}O\textsubscript{2}. We then supplemented LA to growth medium and observed a partial rescue of growth phenotype, especially when administered after exposure to H\textsubscript{2}O\textsubscript{2}, confirming that the beneficial effect of LA may be primarily driven by its antioxidant function, as previously observed.\textsuperscript{25} Thus, LA could be a viable therapeutic option for MECR patients, even if it does not compensate directly for the lack of protein lipoylation typical of mtFAS dysfunction. Appropriate clinical trials titrating LA in patients are needed.

Remarkably, this report on MECR pairs the recent observation of an LHON-like phenotype associated with biallelic variants in MCAT, also involved in the mtFAS pathway. The knockdown in human cells of either MCAT or MECR leads to mitochondrial dysfunction characterised by impaired assembly of respiratory complexes, mainly due to the loss of acyl-NDUFAB1 and its interaction with LYRM proteins.\textsuperscript{26} Moreover, pathogenic variants in both genes have been associated with a severe childhood-onset oxidative phosphorylation defect, also characterised by reduced levels of intact complexes/supercomplexes in the MCAT described case.\textsuperscript{25} Conversely, we failed to observe such a drastic downstream consequence of mutant MECR on OXPHOS complexes and respiratory function in our patient-derived primary cultures of fibroblasts. As mentioned, the residual activity of MECR in these two patients may be sufficient to maintain a basal mitochondrial respiration, which might well be defective in neurons like the retinal ganglion cells. Consistently, the phenotype of our patients is mild, limited to optic atrophy, whereas the full-blown encephalopathy described in the other reported patients broadly belongs to the spectrum of Leigh syndrome.\textsuperscript{13} Indeed, the dual clinical presentation of Leigh syndrome and subacute optic neuropathy is now described for the complex I-related genes DNAJC30 and NDUFAS2 and possibly NDUFAP12, as well as the mtFAS-related MCAT and MECR, suggesting that this phenotypic spectrum could be common to different defects in mitochondrial enzymes. On the contrary, even the common mtDNA mutations associated with LHON may occasionally lead to Leigh syndrome, remarking the mechanistic link between these two clinical phenotypes of mitochondrial disease.\textsuperscript{28,29}

Overall, the dysfunctional mtFAS pathway emerges as a previously overlooked pathogenic mechanism reflecting on mitochondrial function, in addition to the already proposed protein lipoylation defect, which possibly contributes to the mitochondrial respiratory impairment typical of mtFAS-linked disease.\textsuperscript{13} In conclusion, mtFAS impairment is a new pathogenic mechanism leading to LHON-like recessive optic neuropathy, in addition to the previously described cases with more severe Leigh-like encephalopathic involvement. Further studies on patient-derived neuronal cells are needed to clarify the consequences of MECR mutations associated with the LHON-like phenotype in the cells targeted by this disease. However, the beneficial effect of LA on oxidative stress suggests that this condition is possibly amenable to treatment.

Acknowledgements The authors are deeply grateful to the patients who participated to this work.

Contributors Conceptualisation: LC, EB, PB, VC, CF. Data curation: CF, AD, MLC, CVT. Formal analysis: CF, AD, MLC, CVT, CLM, MB, DO, FP, MC, FB. Funding acquisition: EB, LC. Investigation and methodology: all authors. Software: AT, SEH, LA, JL, DK, FC, JME. Writing—original draft: CF, AD, MLC, CVT, CLM, VC, AM, PB, EB, LC. Guarantor: LC. Writing—review and editing: all authors.

Funding The research activity was supported by the Italian Ministry of Health grant GR-2016-02361449 to LC and ‘Ricerca Corrente’ funding to VC and AM.

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Comitato Etico di Area Vasta Emilia Centro della Regione Emilia-Romagna (CE-AVEC), #2112018/SPER/AUSLBO. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

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