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BRIEF COMMUNICATION

A Novel *NDUFS3* mutation in a Chinese patient with severe Leigh syndrome

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Abstract

Leigh syndrome is one of the most common subtypes of mitochondrial disease. Mutations in encoding genes of oxidative phosphorylation complexes have been frequently reported, of which, *MTATP6* was one of the most frequently reported genes for Leigh syndrome. In this study, by using next-generation sequencing targeted to MitoExome in a patient with clinical manifestations of Leigh syndrome, two missense mutations of *NDUFS3* (c.418 C > T/p.R140W and c.595 C > T/p.R199W) were identified, of which c.418 C > T was novel. Functionally, the patient derived lymphoblastoid cells showed decreased amount of NDUFS3 and complex I assembly when compared with two control cells. Although *NDUFS3* mutations have been related to late onset Leigh syndrome, we found that the patient carrying these two mutations developed an early onset Leigh syndrome. To our knowledge, this is the second study on patient carrying *NDUFS3* mutations. In conclusion, we identified a novel Leigh syndrome causing *NDUFS3* mutation and expanded the clinical spectrum caused by *NDUFS3* mutations in this study.

Introduction

Leigh syndrome (LS; Online Mendelian Inheritance in Man [OMIM] 25600) is one of the most common subtypes of mitochondrial disease characterized by developmental delay, ataxia, progressive loss of movement abilities, and visible symmetrical lesions in basal ganglia via magnetic resonance imaging (MRI) [1]. LS is caused by mutations in either subunit genes of oxidative phosphorylation system (OXPHOS) or regulatory genes of OXPHOS assembly and function maintenance [2]. To date, over 75 LS causing genes in both nuclear DNA and mitochondrial DNA (mtDNA) have been identified [3]. However, many LS patients were genetically undiagnosed, indicating that many disease causing gene mutations remain unidentified.

As a major rate-limiting multimeric enzyme of OXPHOS, complex I consists of 45 subunits and is the largest complex in all five OXPHOS complexes. Therefore, it is not surprising that complex I mutations are the leading cause of LS. To date, mutations in over twenty complex I related genes have been reported to cause LS [2], however, the spectrum of complex I is still incomplete. In this study, we performed mutation screening in patients with typical clinical manifestations of LS. In one patient, we identified a novel missense mutation in *NDUFS3*. The pathogenesis of this novel mutation was verified by determining the mitochondrial functions in patient derived lymphoblastoid cell lines.

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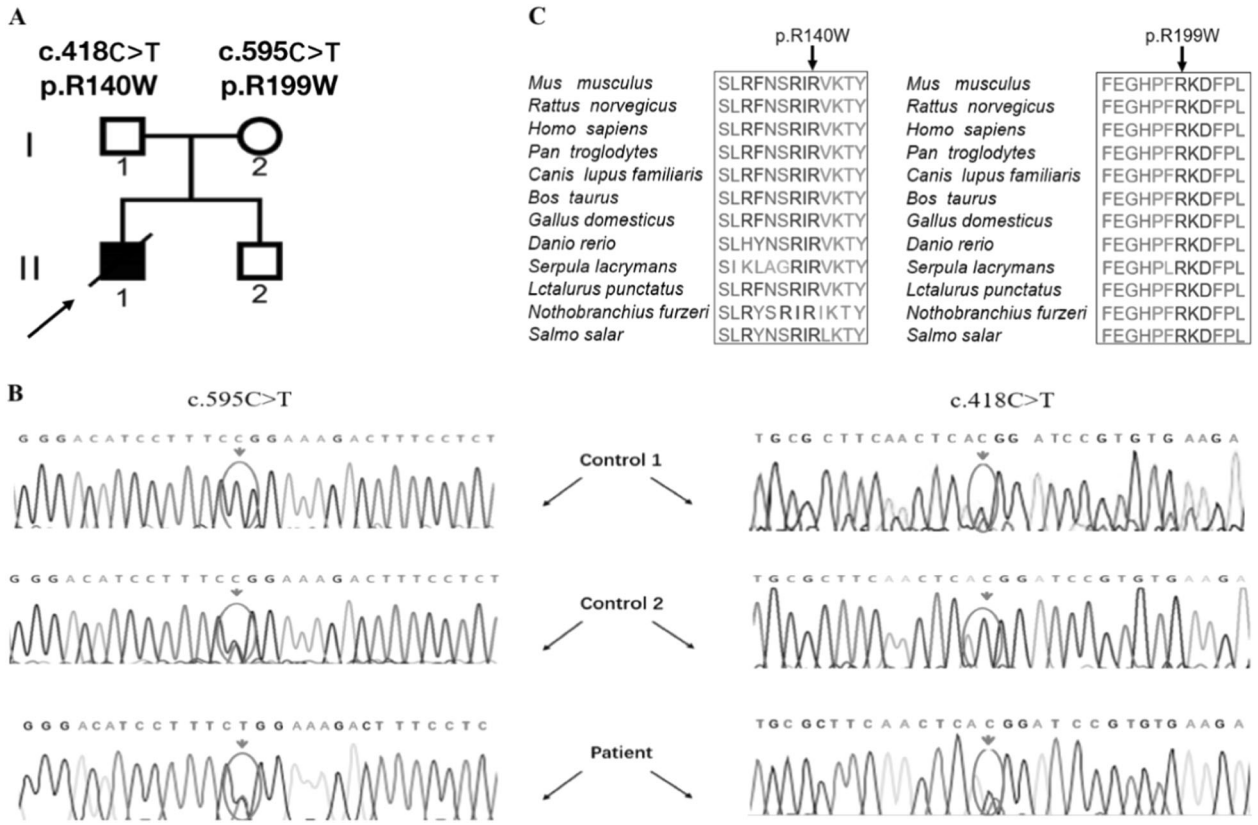

Fig. 1 Family pedigree and mutation analysis of *NDUFS3*. **a** The proband was indicated by black arrows. **b** Sequencing chromatograms of *NDUFS3* in the patient and the parents of the patient. Control 1, father; control 2, mother. **c** Conservation analysis of *NDUFS3* c.418 C > T (p.R140W) and c.595 C > T (p.R199W) using Clustal W. Sequences of *Mus musculus* (NP_080964.1), *Rattus norvegicus* (NP_001099959.1), *Homo sapiens* (NP_004542.1), *Pan troglodytes* (NP_001073382.1), *Canis lupus familiaris* (XP_533185.2), *Bos taurus* (NP_777244.1), *Gallus domesticus* (NP_001139804.1), *Danio rerio* (NP_001017755.1), *Serpula lacrymans* (XP_007312158.1), *Ictalurus punctatus* (NP_001188173.1), *Nothobranchius furzeri* (XP_015809492.1), *Salmo salar* (XP_014030569.1) were download from National Center for Biotechnology Information

Material and methods

Patient

The patient was born in a non-consanguineous family and was clinically diagnosed with LS at his age of 7 months (died at 2 years of age). He was referred to Peking University First Hospital due to torticollis. A first brain MRI, performed at 7 months, showed a high T2 signal intensity in the white matter of hemispheres, basal ganglia, and brain stem of the patient. A second MRI of the patient was performed at 12 months and showed a much severe disease progression. In the mean while, organic acid screening of urine using Liquid chromatography–mass spectrometry revealed a broad changes of metabolites such as lactic acid, acetylformic acid, phosphoric acid, and malic acid, of which lactic acid, pyruvic acid, and malic acid are closely associated with OXPHOS disorders. Moreover, the patient was found presented symptom of metabolic acidosis due to the decreased blood potential of hydrogen (pH) (blood pH = 7.12, normal 7.35–7.45). The study protocol was approved by the Ethics Committee of Peking University First Hospital, China.

Sequencing and functional validation

Next generation sequencing targeted to MitoExome was performed using Illumina HiSeq 2000 sequencer as described previously [4]. Illumina clean reads were aligned to each human reference genome (GRCh38, UCSC) using the BWA program and quality scores were recalibrated and realigned to reference using the GATK software package. Pathogenicity of the variants were evaluated through the filters such as allele frequency, effects on protein functions, and consistent with a recessive model of pathogenesis [4]. B-lymphocytes obtained from the patient and his healthy parents were immortalized as described previously [5]. Blue Native PAGE/Immunoblot analysis and in gel activity assay of OXPHOS complex I and IV were performed according to standard procedures [1].

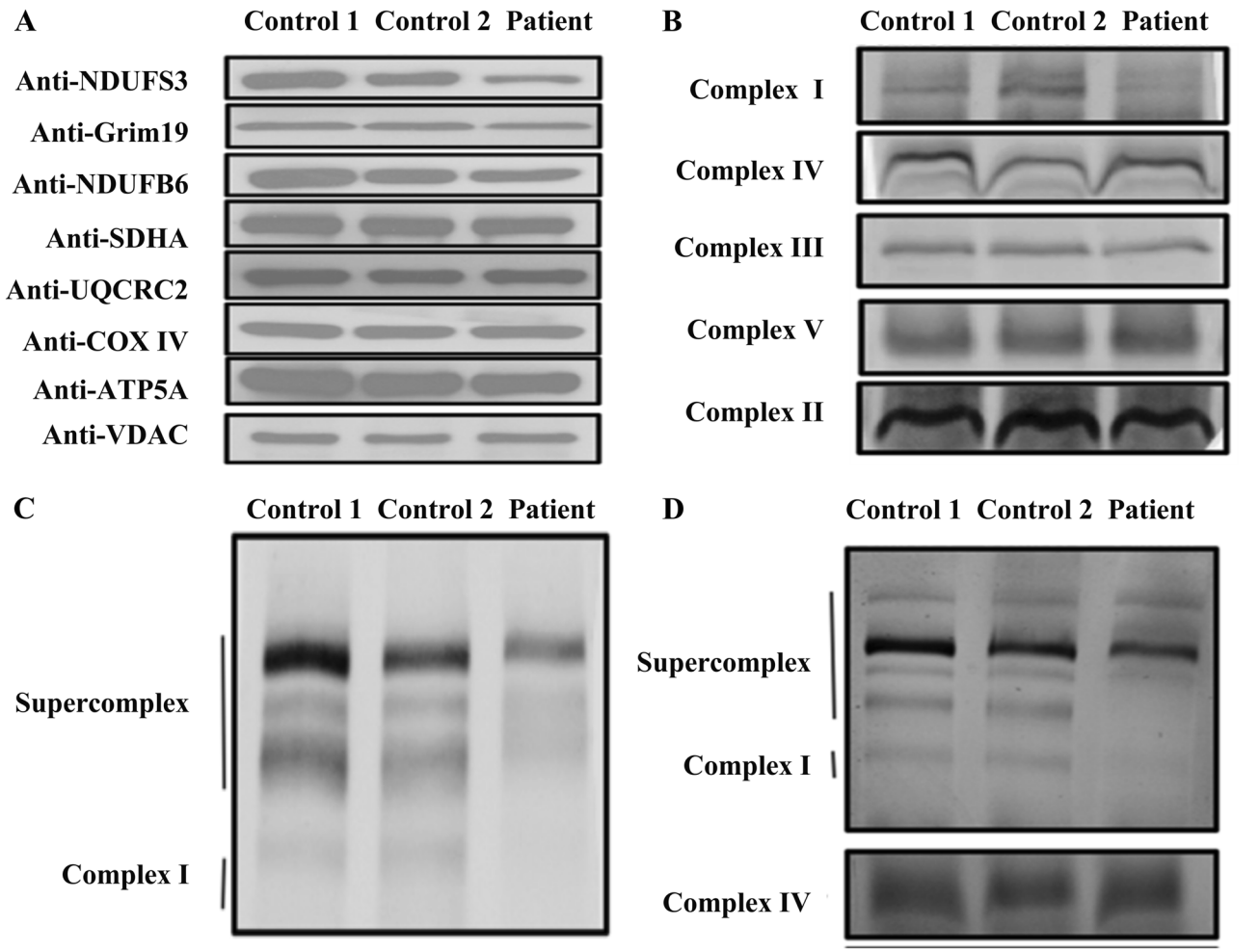

Fig. 2 Decreased level of *NDUFS3* associated with complex I and complex I containing supercomplex assembly. **a** Whole cell extraction from the patient and two control cells were solubilized with RIPA (Cell Signaling Technology, Danvers, MA) and then subjected to Sodium dodecyl sulfate PAGE/immunoblot analysis. VDAC was used as total loading control. **b** Mitochondrial protein from the patient and two control cells were extracted by 10% n-dodecyl β-D-maltoside (Sigma, St. Louis, MO, USA) and then subjected to Blue native PAGE/immunoblot analysis. Complex I, complex II, complex III, complex IV and complex V were immunoblotted with anti-Grim19 (1:1,000; MitoSciences, Eugene, OR, USA), SDHA (1:1,000), core2 (1:1,000; MitoSciences), COX IV (1:1,000; MitoSciences), and ATP-5A (1:1,000; MitoSciences) antibodies, respectively. **c** Mitochondrial protein from the patient and two control cells were extracted by digitonin (Sigma) with a detergent/protein ratios at 6 g/g. Complex I was detected by anti-Grim19 (1:1, 1000). **d** In-gel activity assays of complex I and complex IV were performed after Blue native PAGE in **c**

Results and discussion

To identify the disease causing gene mutations, exons of 1033 nuclear-encoded mitochondrial genes and entire mitochondrial genome from the peripheral blood leukocytes of the patients were captured and sequenced. Following the established variant prioritization criteria [4], we identified two candidate complex heterozygous mutations of *NDUFS3* in the patient. These two mutations, c.418 C > T (exon 5) and c.595 C > T (exon 6), were further confirmed by Sanger sequencing (Fig. 1a, b). The former missense mutation resulted in an amino acid change from arginine to tryptophan at the 140th position (p.R140W), which was not reported by in-depth search of the databases such as ExAC, gnomAD, LOVD, and ClinVar, whereas the latter missense mutation (p.R199W) has been reported to cause LS in 2004 [6]. Notably, both amino acid residue were highly conserved among different species (Fig. 1c).

To validate the pathogenic role of c.418 C > T (p.R140W) and c.595 C > T (p.R199W), expression of NDUFS3 and the mitochodndrial functions were tested in immortalized lymphocytes derived from the patient and his parents. As shown in Fig. 2a, expression of NDUFS3 decreased in patient derived immortalized lympyocytes compared with that of two control cells, while expression of other OXPHOS subunits such as Grim19 (NDUFA13), NDUFB6, SDHA, UQCRC2, COXIV, and ATP5A were not affected. Previous study showed that NDUFS3 with two mutations, c.434 C > T and

c.595 C > T, was more prone to aggregation than its wild type [7]. We speculated that the novel c.418 C > T may have the same effect, since it is very close to c.434 C > T. Furthermore, Blue Native PAGE/Immunoblot analysis revealed a decreased level of OXPHOS complex I and complex I containing supercomplex in the patient derived lympyocytes compared with that of two control cells (Fig. 2b, c). Consistently, in gel activity assay of OXPHOS complexes showed a dramatic decrease of complex I activity in the patient cells compared with that of two control cells (Fig. 2d). Therefore, our results indicated that in the patient, mutations of c.418 C > T and c.595 C > T decreased protein integrity and impaired complex I and complex I containing supercomplex assembly.

Although mutations in *NDUFS3* were first reported at 2004 [6], few mutations were reported so far. Suggesting that *NDUFS3* is not a mutation hotspot in patients with mitochondrial disease. In this study, we found the patient diagnosed with late onset LS reported in 2004 exhibited an abnormal psychomotor development when he was nine years old, while the patient in this study developed an early onset LS. Since the degree of complex I impairment was not performed in patient reported at 2004, it is not clear if the degree of complex I impairment or other features such as population genetic backgrounds contributed to the varied disease severity.

In conclusion, we reported a patient with LS carrying c.418 C > T and c.595 C > T in *NDUFS3*. To our knowledge, this is the second study on *NDUFS3* mutation, and for the first time, we found mutations in *NDUFS3* can cause LS in infant.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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