

SHORT COMMUNICATION

The mitochondrial DNA 10197 G > A mutation causes MELAS/Leigh overlap syndrome presenting with acute auditory agnosia

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Abstract

Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes/Leigh (MELAS/LS) overlap syndrome is a mitochondrial disorder subtype with clinical and magnetic resonance imaging (MRI) features that are characteristic of both MELAS and Leigh syndrome (LS). Here, we report an MELAS/LS case presenting with cortical deafness and seizures. Cranial MRI revealed multiple lesions involving bilateral temporal lobes, the basal ganglia and the brainstem, which conformed to neuroimaging features of both MELAS and LS. Whole mitochondrial DNA (mtDNA) sequencing and PCR-RFLP revealed a *de novo* heteroplasmic m.10197 G > A mutation in the NADH dehydrogenase subunit 3 gene (*ND3*), which was predicted to cause an alanine to threonine substitution at amino acid 47. Although the mtDNA m.10197 G > A mutation has been reported in association with LS, Leber hereditary optic neuropathy and dystonia, it has never been linked with MELAS/LS overlap syndrome. Our patient therefore expands the phenotypic spectrum of the mtDNA m.10197 G > A mutation.

Keywords

Cortical deafness, mitochondrial disorders, NADH dehydrogenase subunit 3 gene

History

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Introduction

Mitochondrial disorders comprise a large group of diseases that manifest with heterogeneous clinical features. The most common subgroups include mitochondrial encephalomyopathy with lactate acidosis and stroke-like episodes (MELAS), Leigh syndrome (LS), Leber hereditary optic neuropathy, chronic progressive external ophthalmoplegia and myoclonic epilepsy with ragged red fibers. While mitochondrial DNA (mtDNA) mutations are the main genetic defects that are causative of MELAS, both mtDNA and nuclear gene mutations are responsible for LS (Koopman et al., 2012).

Clinically, patients presenting with an overlap of different subtypes of mitochondrial disorders are rare (Crimi et al., 2003; Naini et al., 2005; Pulkes et al., 1999; Wilichowski et al., 1998). For example, MELAS/LS overlap syndrome, a particular subtype of mitochondrial disorders with clinical and magnetic resonance imaging (MRI) features that are characteristic of both MELAS and Leigh syndrome, has been documented in only 10 patients to date (Blok et al., 2007; Crimi et al., 2003; Lim et al., 2009; Liolitsa et al., 2003; Wang et al., 2010). MELAS/LS overlap syndrome is associated with the m.13513 G > A mutation in the mitochondrial NADH dehydrogenase subunit 5 gene (*ND5*) as well as three other mutations in *ND5* and *ND3* (Crimi et al., 2003; Lim et al., 2009; Liolitsa et al., 2003; Wang et al., 2010).

Here, we report a case of MELAS/LS overlap syndrome presenting with acute auditory agnosia and seizures caused by a *de novo* m.10197 G > A mutation in *ND3*. While this mutation has been reported in patients with LS and Leber hereditary optic

neuropathy (Kirby et al., 2004; Sarzi et al., 2007), no reports have previously associated it with MELAS/LS overlap syndrome.

Materials and methods

Case report

The patient is a 14-year-old adolescent who experienced an unremarkable birth and development. He is the second child of a healthy mother and his family medical history is negative. One month previously, he suddenly developed tinnitus of the left ear, acousma and headache. Subsequently, he suffered progressive hearing loss and showed little response to the speech of others. One week after the disease onset, he developed several generalized seizures and was admitted to the local hospital where a brain MRI showed abnormal signals in the bilateral basal ganglia, midbrain and the right temporal lobe (Figure 1a–d). A lumbar puncture, serum ceruloplasmin measurement and otological examination of his ears showed no remarkable findings. His seizure was controlled by antiepileptic drugs but there was no improvement in his hearing, so he was referred to our hospital one month after disease onset.

A physical examination revealed that the patient was of a normal height (168 cm) and weight (62 kg) for his age, but a neurological examination showed severe cortical deafness, which deprived him of the ability to communicate orally. He could not understand voices and words, but could still recognize the sound of music and the ticking of a clock. His ability of written communication was intact. There were no other focal neurological signs except for slight central facial palsy on the left side. An ophthalmic fundus examination gave negative result. Blood lactate levels were slightly elevated (2.5 mmol/L, normal <2.0 mmol/L). A second brain MRI revealed additional lesions in the bilateral temporal lobes (Figure 1e and f).

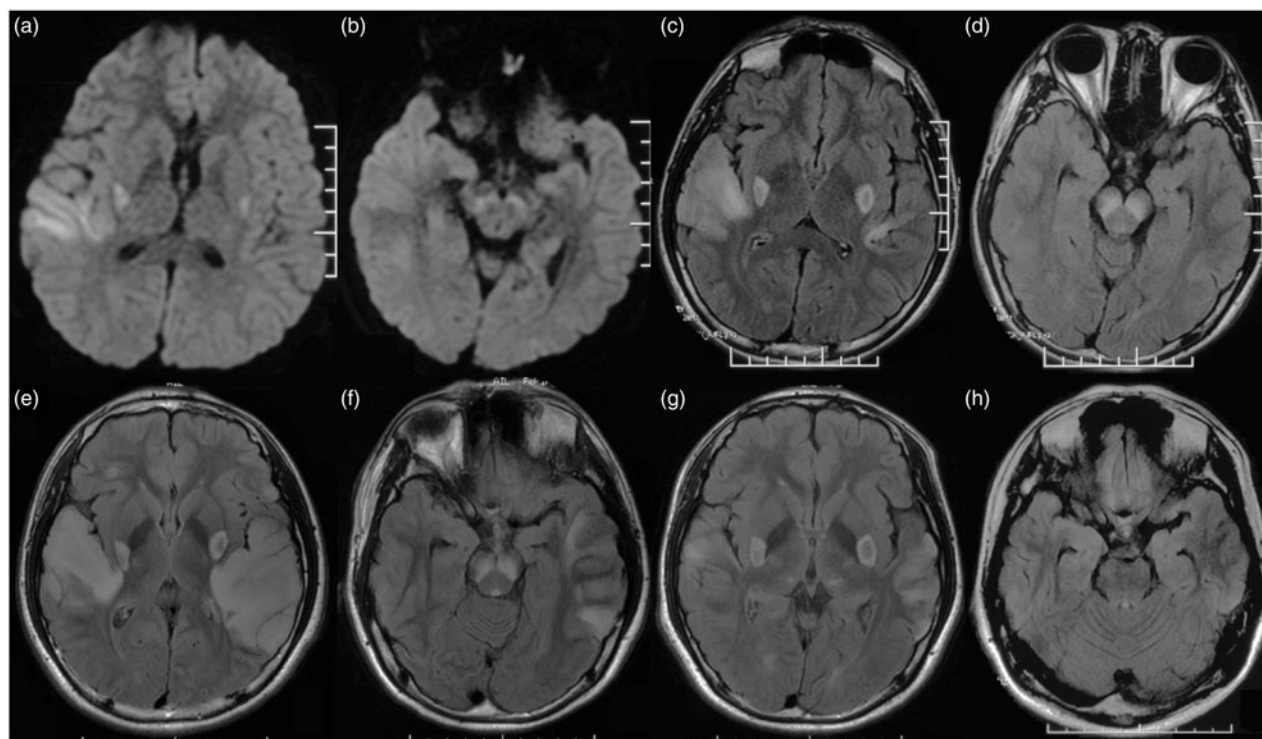


Figure 1. Brain MRI of the patient taken one week (a–d), one month (e, f) and three months (g, h) after disease onset. Both diffusion weighted imaging (a, b) and T2 weighted imaging (c, d) showed high signals in the right temporal lobe, bilateral basal ganglia, and midbrain shortly after disease onset. One month later, a new lesion developed in the contralateral temporal lobe in addition to the old lesions (e, f). Three months after disease onset, the lesions were alleviated both in the temporal lobes (g) and in the midbrain (h).

The patient underwent detailed auditory examinations. Pure tone audiometry showed a normal auditory threshold in both ears (25 dBHL for the left ear and 20 dBHL for the right). Acoustic immittance audiometry and otoacoustic emission testing were unremarkable. However, the speech recognition threshold could not be defined in speech audiometry, and the word recognition score at 50 dBHL was 0% in the left ear and 20% in the right. Consistently, the brainstem auditory evoked potential (BAEP) showed normal latencies in both ears, while the cognitive evoked potential P300 was absent.

Following antiepileptic therapy and treatments including vitamin B and several coenzymes to improve the function of his mitochondrial respiratory chain, the patient had no more seizures but his cortical deafness recovered very slowly. A follow-up MRI examination performed three months after the disease onset showed alleviation of the lesions in both the temporal lobes and the brainstem to some extent (Figure 1g and h). A muscle biopsy was performed of the patient, and molecular genetic analysis was carried out on both the patient and his mother with their informed consent.

Muscle pathology examination

Muscle specimens were snap frozen in pre-cooled isopentane, and stored at -80°C until analysis. Cryostat sections were prepared and stained with hematoxylin–eosin, modified Gomori trichrome, cytochrome C oxidase, succinate dehydrogenase and other routine histochemical methods.

mtDNA mutation analysis

Total DNA was extracted from skeletal muscle, blood leukocytes and urine sediment of the patient using standard methods (Ma et al., 2009). Whole mtDNA genome sequencing analysis was performed on muscle DNA to exclude common mtDNA mutations including m.3243 A>G, m.8344 A>G, m.8993 T>G/

C and large-scale deletions (Zhao et al., 2011). The mitochondrial gene sequencing data were compared with human mitochondrial genome databases MITOMAP (<http://www.mitomap.org/MITOMAP>) and mtDB (<http://www.genpat.uu.se/mtDB>).

DNA samples of all three tissues were analyzed by PCR-RFLP to quantify the m.10197 G>A mutation load. A 650 bp fragment was PCR amplified using the primer pair 5'-TCTATTGATGAGGGTCTTACT-3' (light strand 9973–9993) and 5'-GGTGTGAGGGTTATGAGAGT-3' (heavy strand 10,622–10,602) with an initial denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. The PCR product was digested with *Cac8I*, which cleaves the wild-type 650 bp fragment into two fragments of 227 and 423 bp, while the m.10197G>A mutation eliminates this restriction site. The ratio of digested versus undigested DNA fragments was determined using a densitometer and Gel-Pro Analyzer software (Media Cybernetics, Bethesda, MD). Total DNA from the blood leukocytes and urine sediment of the patient's unaffected mother was also extracted and analyzed by PCR-RFLP. To rule out the possibility of low levels of m.10197G>A mutation, DNA samples of the mother's leukocytes and urine sediment were further analyzed by next-generation sequencing using mitochondria enrichment kit (MitoCap™, MyGenostics, Beijing, China) and Illumina HiSeq2000 sequencing platform. The 100bp-long mitochondria enrichment probes were designed to tile along the 16,569 bp mitochondrial genome. The enrichment experiment was conducted according to the manufacturer's protocol (www.mygenostics.com), and then the enriched library was paired-end 100 bp sequenced on Illumina HiSeq2000 sequencer.

Results

Analysis of muscle pathology revealed little was abnormal except for some darkly stained succinate dehydrogenase-positive

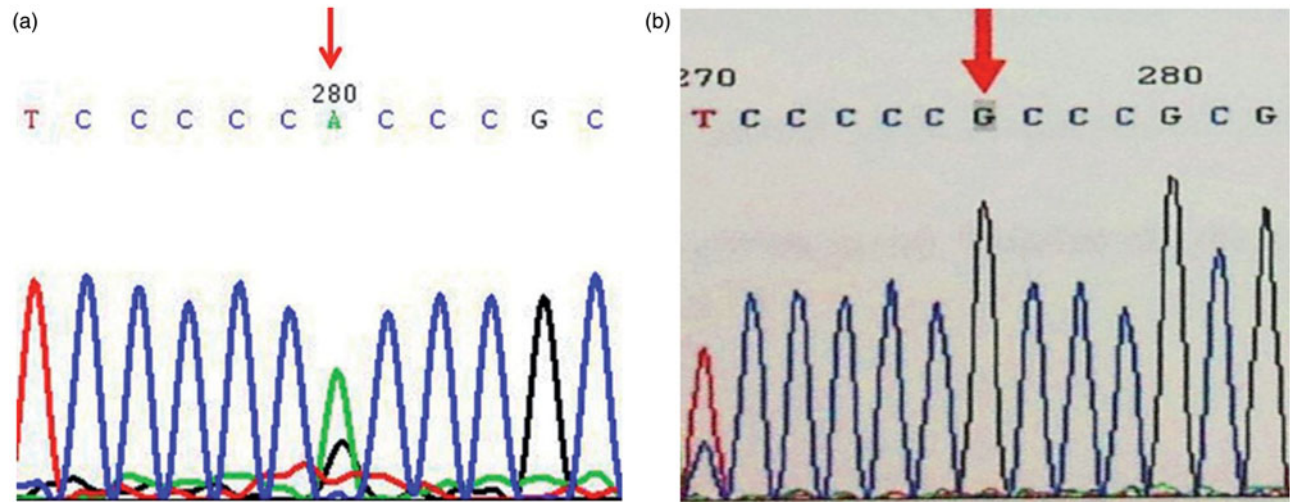
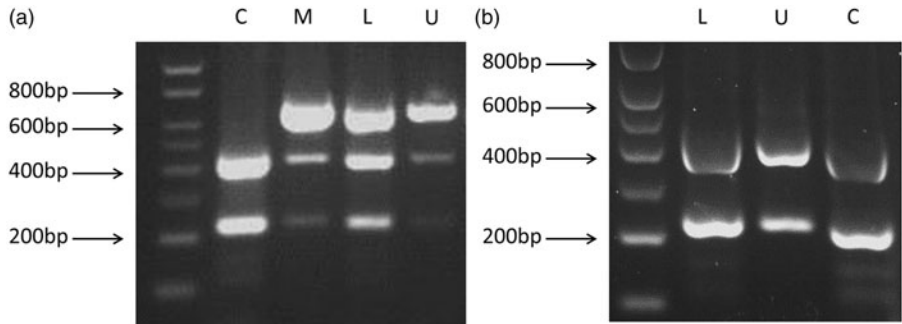


Figure 2. Mitochondrial DNA sequencing identified a heteroplasmic m.10197 G>A mutation in the skeletal muscle of the patient (a) and wild-type m.10197G in a normal control (b).

Figure 3. PCR-RFLP analysis of the patient and his mother. (a) The m.10197 G>A mutation was detected in the patient's skeletal muscle (M), blood leukocytes (L), and urine sediment (U) by PCR-RFLP. (b) This m.10197 G>A mutation was absent from both the leukocytes (L) and urine sediment (U) of the patient's mother. C, normal control.



perimysial blood vessels. Neither ragged-red nor COX-negative fibers were observed.

Whole mtDNA sequencing identified a heteroplasmic m.10197G>A mutation in the skeletal muscle of the patient (Figure 2), which was further confirmed by PCR-RFLP. The mutation loads were 85.1%, 61.1%, and 75.8% in skeletal muscle, leukocytes, and urine sediment, respectively (Figure 3a). The mutation was not detected in the blood or urine of the patient's mother by PCR-RFLP (Figure 3b). Next-generation sequencing showed that m.10197 was all read as reference base G for 1322 times both in her leukocytes and urine sediment, with no mutation base found.

Discussion

Our patient presented with cortical deafness, seizures, episodic headaches, and infarct-like lesions in the cerebral cortex, which suggested a diagnosis of MELAS. Meanwhile, his brain MRI showed symmetrical lesions of the bilateral basal ganglia and midbrain, which is typical of LS. These clinical and neuroimaging characteristics together resulted in a diagnosis of MELAS/LS overlap syndrome. Although muscle pathology did not provide strong supportive evidence for mitochondrial disease, we nevertheless undertook whole mtDNA sequencing considering the typical clinical manifestation of the patient.

MtDNA sequencing revealed a heteroplasmic m.10197G>A mutation in ND3, which was confirmed by PCR-RFLP. This mutation was previously reported to be associated with mitochondrial disorders (Chae et al., 2007; Kirby et al., 2004; Sarzi et al., 2007; Wang et al., 2009), and changes the evolutionarily conserved amino acid 47 from alanine to threonine. This substitution is located in an extramembrane loop connecting

two putative transmembrane domains of ND3 and is associated with reduced activity of respiratory chain enzyme complex I (Liu et al., 2012; Nesbitt et al., 2012; Sarzi et al., 2007; Taylor et al., 2001). We also considered this mutation to be pathogenic in the present case for three reasons: (1) the mutation is heteroplasmic at various levels in different tissues, with the highest level in postmitotic tissue (skeletal muscle); (2) mtDNA sequencing ruled out other pathogenic mutations; (3) the mutation was not found in the unaffected mother or normal controls. It is conceivable that this is a *de novo* mutation in the patient because it was absent in both the blood and urine sediment of his mother, although germline mosaicism cannot be ruled out. However, the mutation was also reported to be a neutral polymorphisms in Polynesians (Kirby et al., 2004). Future functional studies should be carried out to strengthen its pathogenicity in this patient.

To our knowledge, this is the first reported case of MELAS/LS overlap syndrome caused by the mtDNA m.10197 G>A mutation. As summarized in Table 1, other researchers have demonstrated its association with LS, and Leber hereditary optic neuropathy and dystonia (LDYT) (Chae et al., 2007; Kirby et al., 2004; Sarzi et al., 2007; Wang et al., 2009). Both our patient and those previously reported in the literature had lesions in their basal ganglia and brainstem. However, our patient presented with some characteristics that differed from earlier cases; for example, a later disease onset without maternal inheritance, the significant involvement of the cerebral cortex, and the absence of dystonia and optic neuropathy. As only seven months passed from the disease onset to the last telephone follow-up of our patient, further clinical observations should be made to determine whether he will develop dystonia or optic neuropathy at a later stage.

Additionally, the mutation loads of m.10197 G>A were more than 80% in the skeletal muscle and more than 60% in mitotic

Table 1. Patients with the mtDNA m.10197 G>A mutation reported in the literature.

Patient No.	Gender	Age of onset	Age at death	Phenotype	Brain lesions	Tissue	Mutation load (%)	References
1	M	14 yr	Alive at 14 yr	MELAS/LS overlap	Cortex, basal ganglia and brainstem	Skeletal muscle Urine Sediment Leukocytes	85.1 75.8 61.1	Present study
2	F	2 yr	Alive at 13 yr	LS-like	NA	NA	NA	Kirby et al. (2004) Sarzi et al. (2007)
3*	F	14 yr	Alive as adults	LS and dystonia	NA	Leukocytes	67	
4	M	1 mon	5 mon	LS and dystonia	Brainstem	Skeletal muscle	100	Kirby et al. (2004) Sarzi et al. (2007)
5	M	2 mon	2 mon	LS and dystonia	Basal ganglia	Liver	100	
6	F	2 mon	8 mon	LS and dystonia	NA	Leukocytes	100	Kirby et al. (2004) Sarzi et al. (2007)
7†	M	4 mon	11 mon	LS and dystonia	Basal ganglia, thalamus and brainstem	Skeletal muscle	100	
8	M	5 mon	Alive at 5 yr	LS and dystonia	Basal ganglia	NA	NA	Kirby et al. (2004) Sarzi et al. (2007)
9‡	F	6 yr	Alive as adults	LS and dystonia	NA	Skeletal muscle	100	
10	F	5 mon	Alive at 5 yr	LS	NA	Fibroblasts	96	Kirby et al. (2004) Sarzi et al. (2007)
11	M	NA	2 yr	LS	NA	Skeletal muscle	74	
12	M	5 mon	11 mon	LS and dystonia	Basal ganglia, thalamus and brainstem	Leukocytes	100	Kirby et al. (2004) Sarzi et al. (2007)
13¶	F	7 yr	Alive at 9 yr	Dystonia	NA	Leukocytes	100	
14	M	4 yr	Alive at 9 yr	Dystonia	Basal ganglia	Skeletal muscle	98	Chae et al. (2007)
15	M	3 mon	Alive at 1 yr	Dystonia	Basal ganglia	Skeletal muscle	86	
16§	M	5 yr	Alive as adults	Solitary stroke	Basal ganglia and brainstem	Skeletal muscle	80	Wang et al. (2009)
17	M	8 yr	Alive as adults	LDYT	Basal ganglia	Leukocytes	100	
18	F	8 yr	Alive as adults	LDYT	Basal ganglia	Leukocytes	100	Wang et al. (2009)
19	F	5 yr	Alive as adults	LDYT	NA	Leukocytes	100	
20	F	8 yr	Alive as adults	LDYT	NA	Leukocytes	100	Wang et al. (2009)
21	M	5 yr	Alive at 18 yr	LDYT	NA	Leukocytes	100	

MELAS, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes. LS, Leigh syndrome. LDYT, Leber hereditary optic neuropathy and dystonia. NA, not available.

*Mother of patient 4, 5, 6.

†Brother of patient 8.

‡Mother of patient 10, 11, 12.

¶Sister of patient 14.

§Family member of patient 17–21, there were another 10 members of his family presented as optic atrophy without dystonia, not shown here.

tissues (leukocytes) of our patient, while most of the previously reported patients with the same mutation harbored 100% mutation loads in their skeletal muscle or leukocytes (Chae et al., 2007; Kirby et al., 2004; Sarzi et al., 2007; Wang et al., 2009). This difference could explain the observed phenotypic differences, including the brain-only involvement, relatively mild symptoms, and late onset of our patient, in contrast to reported cases with an earlier onset and multi-organ involvement. It is known that the mtDNA mutation load must exceed a certain threshold to cause mitochondrial dysfunction (Sarzi et al., 2007). We therefore deduced that m.10197 G>A must have a high threshold because all patients with this mutation had a high mutation load. This is quite different from other mitochondrial-encoded ND mutations. For example, a low mutation load of m.13513 G>A in ND5 below 50% is sufficient to cause severe complex I deficiency (Wang et al., 2010). As the m.10197 G>A mutation can be associated with various clinical syndromes, this indicates that the mitochondrial phenotype may be determined by multiple factors other than mtDNA mutation type, the degree of heteroplasmy, and threshold effect (Werner et al., 2012). The mitochondrial DNA haplotype, nuclear genetic background, and environmental factors might also play a role in the heterogeneous presentation of a mitochondrial molecular defect.

Auditory agnosia was a prominent clinical feature in the patient of the present study. Although hearing impairment is common in mitochondrial diseases, especially in MELAS, most patients show isolated or combined sensorineural hearing loss, which indicates a predominantly cochlear origin of the hearing deficit (Chinnery et al., 2000). In this case, detailed audiological evaluation revealed that only the temporal lobes were affected without the involvement of the cochlea. Until now, only cases of MELAS presenting with auditory agnosia from lesions in the bilateral temporal lobes have been reported (Miceli et al., 2008). This study shows that auditory agnosia can also be present in patients with MELAS/LS overlap syndrome.

Declaration of interest

The study was supported by the National Natural Science Foundation of China (No. 30870864 and No. 81341040). The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

- Blok MJ, Spruijt L, M de Coe IF, Schoonderwoerd K, Hendrickx A, Smeets HJ. (2007). Mutations in the ND5 subunit of complex I of the mitochondrial DNA are a frequent cause of oxidative phosphorylation disease. *J Med Genet* 44:e74.
- Chae JH, Lee JS, Kim KJ, Hwang YS, Bonilla E, Tanji K, Hirano M. (2007). A novel ND3 mitochondrial DNA mutation in three Korean children with basal ganglia lesions and complex I deficiency. *Pediatr Res* 61:622–4.
- Chinnery PF, Elliott C, Green GR, Rees A, Coulthard A, Turnbull DM, Griffiths TD. (2000). The spectrum of hearing loss due to mitochondrial DNA defects. *Brain* 123:82–92.
- Crimi M, Galbiati S, Moroni I, Bordoni A, Perini MP, Lamantea E, Sciacco M, et al. (2003). A missense mutation in the mitochondrial ND5 gene associated with a Leigh-MELAS overlap syndrome. *Neurology* 60:1857–61.
- Koopman WJ, Willems PH, Smeitink JA. (2012). Monogenic mitochondrial disorders. *N Engl J Med* 366:1132–41.
- Kirby DM, Salemi R, Sugiana C, Ohtake A, Parry L, Bell KM, Kirk EP, et al. (2004). NDUF5 mutations are a novel cause of lethal neonatal mitochondrial complex I deficiency. *J Clin Invest* 114:837–45.
- Lim BC, Park JD, Hwang H, Kim KJ, Hwang YS, Chae JH, Cheon JE, Kim IO, et al. (2009). Mutations in ND subunits of complex I are an important genetic cause of childhood mitochondrial encephalopathies. *J Child Neurol* 24:828–32.
- Lioltisa D, Rahman S, Benton S, Carr LJ, Hanna MG. (2003). Is the mitochondrial complex I ND5 gene a hot-spot for MELAS causing mutations? *Ann Neurol* 53:128–32.
- Liu YP, Ma YY, Wu TF, Wang Q, Kong QP, Wei XQ, Zhang Y, et al. (2012). Mitochondrial respiratory chain complex I deficiency due to 10191T>C mutation in ND3 gene. *Chin J Contemp Pediatr* 14: 561–6.
- Ma Y, Fang F, Yang Y, Zou L, Zhang Y, Wang S, Xu Y, et al. (2009). The study of mitochondrial A3243G mutation in different samples. *Mitochondrion* 9:139–43.
- Miceli G, Conti G, Cianfoni A, Di Giacopo R, Zampetti P, Servidei S. (2008). Acute auditory agnosia as the presenting hearing disorder in MELAS. *Neurol Sci* 29:459–62.
- Naini AB, Lu J, Kaufmann P, Bernstein RA, Mancuso M, Bonilla E, Hirano M, DiMauro S. (2005). Novel mitochondrial DNA ND5 mutation in a patient with clinical features of MELAS and MERRF. *Arch Neurol* 62:473–6.
- Nesbitt V, Morrison PJ, Crushell E, Donnelly DE, Alston CL, He L, McFarland R, Taylor RW. (2012). The clinical spectrum of the m.10191T>C mutation in complex I-deficient Leigh syndrome. *Dev Med Child Neurol* 54:500–6.
- Pulkes T, Eunson L, Patterson V, Siddiqui A, Wood NW, Nelson IP, Morgan-Hughes JA, Hanna MG. (1999). The mitochondrial DNA G13513A transition in ND5 is associated with a LHON/MELAS overlap syndrome and may be a frequent cause of MELAS. *Ann Neurol* 46:916–19.
- Sarzi E, Brown MD, Lebon S, Chretien D, Munnich A, Rotig A, Procaccio V. (2007). A novel recurrent mitochondrial DNA mutation in ND3 gene is associated with isolated complex I deficiency causing Leigh syndrome and dystonia. *Am J Med Genet A* 143:33–41.
- Taylor RW, Singh-Kler R, Hayes CM, Smith PE, Turnbull DM. (2001). Progressive mitochondrial disease resulting from a novel missense mutation in the mitochondrial DNA ND3 gene. *Ann Neurol* 50:104–7.
- Wang K, Takahashi Y, Gao ZL, Wang GX, Chen XW, Goto J, Lou JN, Tsuji S. (2009). Mitochondrial ND3 as the novel causative gene for Leber hereditary optic neuropathy and dystonia. *Neurogenetics* 10: 337–45.
- Wang Z, Qi XK, Yao S, Chen B, Luan X, Zhang W, Han M, Yuan Y. (2010). Phenotypic patterns of MELAS/LS overlap syndrome associated with m.13513G>A mutation, and neuropathological findings in one autopsy case. *Neuropathology* 30:606–14.
- Werner JH, Peter HGM, Jan AM. (2012). Monogenic mitochondrial disorders. *N Engl J Med* 366:1132–41.
- Wilichowski E, Korenke GC, Ruitenbeek W, De Meirleir L, Hagendorff A, Janssen AJ, Lissens W, Hanefeld F. (1998). Pyruvate dehydrogenase complex deficiency and altered respiratory chain function in a patient with Kearns-Sayre/MELAS overlap syndrome and A3243G mtDNA mutation. *J Neurol Sci* 157:206–13.
- Zhao D, Hong D, Zhang W, Yao S, Qi X, Lv H, Zheng R, et al. (2011). Mutations in mitochondrially encoded complex I enzyme as the second common cause in a cohort of Chinese patients with mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes. *J Hum Genet* 56:759–64.