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A rapid screening with direct sequencing from blood samples for the diagnosis of Leigh syndrome

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ABSTRACT

Large numbers of genes are responsible for Leigh syndrome (LS), making genetic confirmation of LS difficult. We screened our patients with LS using a limited set of 21 primers encompassing the frequently reported gene for the respiratory chain complexes I (ND1–ND6, and ND4L), IV(SURF1), and V(ATP6) and the pyruvate dehydrogenase E1α-subunit. Of 18 LS patients, we identified mutations in 11 patients, including 7 in mDNA (two with ATP6), 4 in nuclear (three with SURF1). Overall, we identified mutations in 61% of LS patients (11/18 individuals) in this cohort. Sanger sequencing with our limited set of primers allowed us a rapid genetic confirmation of more than half of the LS patients and it appears to be efficient as a primary genetic screening in this cohort.

1. Introduction

Leigh syndrome (LS) (OMIM 256000) is an early onset, devastating neurodegenerative disease of the central nervous system (CNS) characterized by symmetrical necrotic lesions in the brainstem, basal

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ganglia and thalamus [1,2]. The symptoms of LS include psychomotor retardation, respiratory difficulties, nystagmus, hypotonia, seizures, myoclonus, ataxia, dystonia, ptosis, ophthalmoplegia and high lactate levels in the blood and cerebrospinal fluid. Mutations in both mitochondrial DNA (mDNA) and nuclear DNA cause LS [3].

LS arises from a deficiency in the enzymes relating to energy production in the mitochondria, such as the respiratory chain complexes I–V, and the pyruvate dehydrogenase complex. Among the enzymes, isolated complex I deficiency is the most frequent oxidative phosphorylation (OXPHOS) defect in children with LS [4,5], followed by a deficiency of complex IV (cytochrome C oxidase) and complex V (ATP synthase). Complex I is composed of seven mDNA encoded NADH dehydrogenase (ND) subunits (ND1–6, ND4L) and at least 38 nuclear DNA subunits [4]. An isolated generalized defect of complex IV is the second most common biochemical abnormalities found in patients with Leigh syndrome [6,7]. *SURF1* mutations, which encode the putative assembly protein of complex IV, have been repeatedly reported [6].

Since a large number of genes are reportedly related to LS, molecular diagnosis appears challenging. However, emerging drugs for LS demand prompt diagnostic confirmation of LS. Although exome sequencing is a powerful method of suspected mitochondrial disorders, it is time and cost consuming, and impractical to be applied to all patients with LS. Based on the reported mutation information, we designed a small set of 21 primers that cover the gene in which LS mutations have been frequently reported [3]. In this study, we have examined the efficacy of our Sanger sequencing method as a genetic screening for LS in 18 unrelated LS cases from one children's hospital. We identified 7 patients with point mutations in mDNA including 2 cases in the *ATP6* gene and five in the *ND* genes. We also elucidated 4 mutations in the nuclear encoded gene, including 3 patients with a mutation in *SURF1* and 1 patient with a mutation in *PDHA1* (pyruvate dehydrogenase E1α-subunit). Our data suggest that Sanger screening using limited sets of primers is useful as first line screening for LS.

2. Methods

We identified 18 patients from 16 families that met the criteria of LS at our institution (2005–2012). Diagnoses of LS were defined as presenting progressive neurologic disease with signs and symptoms of brain stem and/or basal ganglia abnormalities revealed on MR images. The clinical courses are summarized in Table 1 and Supplementary text. We have designed primers encoding mitochondrial derived subunits for complex I (*ND1-6*, *ND4L*) [3]. Primers were also designed on frequently reported gene *SURF 1* from complex IV [7] and *ATP synthase* from complex V [8]. If the blood lactate/pyruvate ratio is less than 10, we first sequenced the *PDHA1* gene (Suppl. Fig. 1) [8]. Methods of genetic analysis, enzyme assays and determination of heteroplasmic rate and associated references are available in the online version of the paper (Suppl. Table 1, Suppl. Table 2, Suppl. text).

3. Results (Table 1, Suppl. Fig. 2)

Of 18 LS patients, we identified gene mutations in 11 patients from 11 families (Table 1, Suppl. Fig. 2). mDNA mutations were identified in 7 patients. An *ND1* mutation of complex I (m3697G>A, p.Gly131Ser) was identified in 2 individuals with homoplasmy. Mutations in *ND3* (m10158T>C, p.Ser34Pro; mutant rate 90% in white blood cell), *ND5* (m13513G>A, p.Asp393Asn; mutant rate 50% in white blood cell) and *ND6* (m14459G>A, p.Ala71Val, homoplasmic state) were identified in a single patient, respectively. One severe patient died at 1 year, and carried a mutation in *ATP6* (m8993T>G, p.Leu156Arg) of complex V of OXPHOS as a homoplasmic state. Instead of T>G, T>C mutation of the same nucleotide, m8993T>C p.Leu156Pro, was observed with homoplasmy in a milder case.

Four patients were identified with mutations in nuclear DNA. *SURF1* mutations were identified in 3 cases, including 2 cases that were compound heterozygous (c.49+1G>T/c.752_753delAG) and (c.574C>T, p.Arg192Trp and c.743C>A, p.Ala248Asp) and 1 case that was homozygous (c.743C>A, p.Ala248Asp). One male patient was identified with a hemizygous mutation (c.121T>C, p.Cys41 Arg) in *PDHA1*. Overall, we identified mutations in 61% of LS patients (11/18 individuals) in this cohort.

4. Discussion

Molecular elucidation of LS at the DNA level is challenging. LS has been associated with a variety of genes in either mitochondrial or nuclear encoded DNA [3]. Surprisingly, we could reveal mutations in 61% of LS patients (11/18 individuals).

We disclosed 7 patients with mDNA mutations. From mitochondrial *ND1*, we identified an m3697G>A mutation in 2 unrelated patients, which has been reported previously in association with mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes (MELAS) [9] and Leber's hereditary optic neuropathy (LHON) [10]. To our knowledge, this is the first report of the m3697G>A/*ND1* gene mutation causing Leigh syndrome. The heteroplasmy rate is reportedly 80% in patients with MELAS (skeletal muscle) and was 56% with LOHN [9,10]. A high mutation load (100%), found in the blood of Patients 1 and 2 may be associated to severe phenotype in our patients [11]. Low level of m3697G>A mutation (~40%) was found in the blood from an asymptomatic mother of Patient 1 (Suppl. Figs. 3 and 4).

For ND3, we found a mutation of m10158T>C with 90% of heteroplasmic rate in one patient showing an early onset and very rapid progress. Severe clinical course and high mutant loads are consistent with reported cases with rapid progression and lethal consequences at early childhood [12]. A mutation of m10158T>C was not detected in the mother of Patient 3 in several tissues examined.

We found one patient with ND5 mutation, m13513G>A which has been described as causing MELAS, LS or overlapping features of the two syndromes [13–15]. We also found one LS patient with m14459G>A/*ND6* mutation that was reported in patients with LHON, dystonia [16] and LS [17]. So far, the phenotype of these two patients is LS without MELAS, LHON.

We found two patients with *ATPase6* mDNA mutations, m8993T>G and T>C, that are frequently reported in the literature [8]. A patient with a T>G mutation usually exhibits earlier onset and more rapid progression compared to T>C mutation at m8993 that was compatible with our patients (Table 1).

We found 4 patients carrying nuclear encoded gene mutations. SURF1 deficiency is the most frequent cause of LS with complex IV (cytochrome C oxidase) deficiency [7]. We identified 3 patients with the *SURF1* mutations [18]. Pyruvate dehydrogenase deficiency (PDH) is a common cause of primary congenital lactic acidosis. The biochemical features of PDH deficiency is elevated blood lactate and pyruvate levels with a normal lactate/pyruvate ratio [19]. According to the genetic screening flowchart for Leigh syndrome (Suppl. Fig. 1), we confirmed 1 patient with a hemizygous mutation in the *PDHA1* gene with 7 sets of primers.

Recently, new drugs such as EPI-743 have been shown to improve neurological and neuromuscular symptoms in LS [20,21]. Rapid genetic confirmation of mitochondrial disease may help initiate such treatment early. Next gene sequencing is revealing a wide range of dual mutations both mitochondrial and nuclear gene from patients with mitochondrial disorders [22–24]. However, it is costly and time consuming. Aiming to elucidate genetic basis of LS patients, we screened with our limited set of primers. Surprisingly, it allowed us confirmation for more than half of the patients. Therefore, this method appears to be efficient as a primary genetic screening. Our data also implicates that LS consisted of few “common” causative genes and a large number of “rare” genes. We are now undertaking whole mDNA and exome sequencing for negative cases of this method [22–24]. These data, together with increasing data of mutations, would help us improve our screening method.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ymgmr.2014.02.006.

Conflict of interest statement

We have no conflict of interest to disclose.

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Table 1
Genetically determined Leigh syndrome in our institution (2005–2012).

Patient	1	2	3	4	5	6	7	8	9	10	11
Age, gender	7 y, M	10 y, F	9 m, F	7 y, M	11 y, M	1 y†, M	2 y, M	4 y, F	9 y, M	25 y†, M	17 y, M
Type of gene	Mito	Mito	Mito	Mito	Mito	Mito	Mito	Nuclear	Nuclear	Nuclear	Nuclear
Gene	*ND1*	*ND1*	*ND3*	*ND5*	*ND6*	*ATPase6*	*ATPase6*	*SURF1*	*SURF1*	*SURF1*	*PDHA1*
Complex	I	I	I	I	I	V	V	IV	IV	IV	
Mutations	m3697G>A (p.G131S) Homo (b)	m3697G>A (p.G131S) Homo (b,s,h,n)	m10158T>C (p.S34P) Hetero (90%) (b)	m13513G>A (p.D393N) Hetero (50%) (b)	m14459G>A (p.A71V) Homo (b)	m8993T>G (p.L156R) Homo (b)	m8993T>C (p.L156P) Homo (b)	c.49+1G>T c.752–753delAG	c.743 C> A p.A248D c.743C> A p.A248D	c.574C>T p.R192 W c.743C>A p.A248D	c.121T>C p.C41R
Consanguinity	N	N	N	N	N	N	N	N	Y	N	N
Inheritance	Maternal* hetero:40%	N.A.	De novo	N.A.	N.A.	N.A.	N.A.	Maternal/ paternal	Maternal/ paternal	N.A.	N.A.
Age at onset	3 y 9 m	3 y 0 m	0 y 5 m	1 y 6 m	2 y 0 m	6 m	1 y 0 m	1 y 7 m	1 y 9 m	2 y	1 y 0 m
Initial Symptoms	Hypertonia Walk regre	Ataxic gait Walk regre Tremor	Hypotonia Strabismus	Dev. delay	Fever → lethargy	Dev. delay/ seizure Hypotonia/ nystagmus	Fever → lethargy	Ataxic gait	Ataxic gait	Dev. delay Ataxia	Dev. delay
Status	Walk Normal class	Wheelchair Special class	Tracheo Mech. venti	Walk	Wheelchair Normal class	(Respiratory failure)	No sitting	Tracheo Mech. venti	Tracheo Mech. venti	(Respiratory failure)	Walk Special school
RC enzymes ↓	I, IV (m)	I, III, IV (m)	I (f)	Normal (m/f)	I, III (m)	I, IV (m)	N.A.	N.A.	IV (f)	IV (m)	N.A.
Morphological findings in muscle	No RRF	No RRF	N.A.	No RRF	RRF	N.A.	N.A.	N.A.	N.A.	RRF	N.A.

MRI											
Basal ganglia hyperintensities	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Brainstem hyperintensities	N	Y	Y	N	N	N	Y	Y	Y	Y	N
Cerebellar atrophy	N	N	N	Y	N	N	N	N	N	Y	Y
Symptoms											
Dysmorphisms	N	N	N	N	N	N	N	Y	Y	N	N
Developmental delay	N	N	Y	Y	N	N	Y	Y	Y	Y	N
Regression	Y	Y	Y	N	N	Y	Y	Y	Y	Y	N
Feeding problems	N	N	Y	N	N	Y	N	N	N	N	N
Ptosis	N	N	N	N	N	N	N	Y	N	N	N
Ophthalmople	N	N	Y	N	N	N	N	Y	N	Y	N
Pyramidal symptoms	Y	Y	Y	Y	Y	N	N	Y	Y	N	Y
Extrapyramidal symptoms	Y	Y	Y	Y	N	Y	N	Y	Y	N	Y
Dystonia	Y	Y	Y	N	N	N	N	Y	Y	N	Y
Hypotonia	N	N	Y	Y	N	Y	Y	Y	Y	N	Y
Ataxia	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y
Neuropathy	N	N	N	N	N	N	N	Y	Y	Y	Y
Others				WPW syndrome		West syndrome					Nystagmus

y: year, m: month, M: male, F: female, mito: mitochondria, Complex: complex in oxidative phosphorylation, b: blood, s: saliva, h: hair, n: nail, RC: respiratory chain, m: muscle, f: fibroblast, RRF: ragged red fibers, N.A.: not analyzed/not determined, N: no, negative, Y: yes, positive, regre: regression, Dev. delay: Developmental delay, Mech.venti: Mechanically ventilated, Ophthalmople: Ophthalmoplegia, *: asymptomatic.

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