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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.