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Biochemical and genetic analysis of Leigh syndrome patients in Korea

Jong-Hee Chae^{a,b}, Jin Sook Lee^b, Ki Joong Kim^b, Yong Seung Hwang^b, and Michio Hirano^{a,*}

^aDepartment of Neurology, Columbia University College of Physicians & Surgeons, 630 West 168th Street, P&S 4-443, New York, NY, USA

^bDepartment of Pediatrics, Seoul National University College of Medicine, Seoul, Republic of Korea

Abstract

Sixteen Korean patients with Leigh syndrome were identified at the Seoul National University Children's Hospital in 2001-2006. Biochemical or molecular defects were identified in 14 patients (87.5%). Thirteen patients had respiratory chain enzyme defects; 9 had complex I deficiency, and 4 had combined defects of complex I + III + IV. Based on the biochemical defects, targeted genetic studies in 4 patients with complex I deficiency revealed two heteroplasmic mitochondrial DNA mutations in ND genes. One patient had the mitochondrial DNA T8993G point mutation. No mitochondrial DNA defects were identified in 11 (68.7%) of our LS patients, who probably have mutations in nuclear DNA. Although a limited study based in a single tertiary medical center, our findings suggest that isolated complex I deficiency may be the most common cause of Leigh syndrome in Korea.

Keywords

Leigh syndrome; Biochemistry; Molecular genetics; Mitochondria; Complex I; Mutation

1. Introduction

Originally described by Dr. Dennis Leigh in 1951, Leigh syndrome (LS) is a progressive neurodegenerative disorder typically beginning in infancy or childhood and is characterized by symmetrical necrosis in the brain stem, basal ganglia, and thalamus [1]. LS is one of the most common mitochondrial disorders and is caused by heterogeneous genetic and biochemical defects, including deficiencies of pyruvate dehydrogenase complex and mitochondrial respiratory chain enzymes. Because respiratory chain enzyme complexes are encoded in mitochondrial DNA (mtDNA) and nuclear DNA (nDNA), LS can be caused by mutations in either genome [2-4]. Little is known about LS in the Korean population. In this study, we characterized the clinical, biochemical, and molecular genetic features of 16 Korean patients with LS evaluated at a major tertiary-care pediatric hospital in Seoul. This is the first report describing the molecular genetic and biochemical features of LS in Korea.

2. Patients and methods

In 2001-2006, 46 patients were evaluated for possible mitochondrial disorders at Seoul National University Childrens' Hospital. All of the patients were screened for other metabolic disorders by analyses of amino acids and organic acids. Sixteen patients were diagnosed with LS based on the following clinical criteria: encephalopathy, psychomotor regression, and characteristic brain magnetic resonance imaging (MRI) with symmetric lesions indicating bilateral necrosis of the basal ganglia, thalamus, and brain stem. None of the patients had MRI findings of central necrosis in the caudate head with involvement of the putamen that are typical of biotin-responsive basal ganglia disease (BBGD) [5]. Using skeletal muscle biopsy samples from all 16 LS patients, we performed histological studies, biochemical measurement of respiratory chain enzyme activities, and mtDNA genetic analyses. Multiple vitamin cocktails were administered to the patients without overt benefits.

2.1. Biochemistry and genetic analysis

We measured the activities of respiratory chain complexes in supernatants from muscle homogenates as previously described [6] in 15 patients out of 16. Total DNA was extracted from muscle using PUREGENE DNA Purification kits (Gentra Systems, Inc., Minneapolis, Minn). Based on the biochemical results, we performed direct sequencing of mitochondrial DNA genes, with BigDye Terminator v3.1 Cycle Sequencing Kits and an ABI PRISM 310 Genetic Analysis (Applied Biosystems, Foster City, CA, USA).

3. Results

3.1. Clinical features

Five male and 11 female patients were analyzed. Most of the patients showed bilateral abnormal T2 high-signal intensities in basal ganglia, brain stem, and/or thalamus. Brain MRS studies were not performed. In the majority of the patients, the initial symptoms were developmental arrest and subsequent regression in the first year of life. Some patients manifested atypical clinical features; two siblings from one family had childhood-onset dystonia, one had a solitary stroke in early infancy, three had infantile spasms, and one developed progressive ataxia with seizures.

Elevated levels of blood lactate were detected in 8 patients (50%). Skeletal muscle biopsy was performed in all of the patients, but most of the samples did not reveal the typical myopathologic features of mitochondrial disorders, such as ragged-red fibers (RRFs) and COX-deficient fibers. We identified strongly succinate dehydrogenase (SDH) positive vessels in two patients, who had mtDNA mutations in NADH dehydrogenase (ND or complex I) genes.

3.2. Biochemical and genetic analysis

Biochemical studies were performed in 15 patients. We identified deficiency of respiratory chain complex I in 9 individuals; combined defects of respiratory chain (RC) complexes I, III, and IV in 4; normal activities in 2. Based on the biochemical results, we sequenced specific mtDNA genes. We identified the causative molecular genetic defects in 5 patients (31.3%). In the 4 patients with combined defects of respiratory enzymes, genetic screening for mtDNA rearrangements, tRNA/rRNA sequencing analysis, and mitochondrial DNA quantitation assay did not reveal any abnormalities. The T8993G mtDNA point mutation, one of the most common causes of LS, was found only in one patient. In the 9 patients with isolated complex I deficiency, we sequenced all seven ND genes encoded by mtDNA. We identified one patient with the previously reported G13513A heteroplasmic mutation in *ND5*, and 3 patients with a novel heteroplasmic G10197A mutation in *ND3* [7].

4. Discussion

We performed the first systematic biochemical and molecular genetic studies of Korean LS patients; defects were identified in 14 out of 16 patients (Table 1). Biochemical analyses of mitochondrial respiratory chain activities in muscle biopsies of 15 patients revealed deficiency of respiratory chain complex I in 9 individuals, combined defects of complexes I, III, and IV in 4, and normal enzyme activities in 2. Among patients with complex I deficiency, one had a previously reported G13513A mtDNA mutation in *ND5* and 3 had a novel pathogenic G10197A in *ND3* [7]. The other 5 patients did not have mtDNA mutations in *ND* genes and therefore are likely to harbor mutations in nDNA. In the 4 LS patients with combined defects of complexes I, III, and IV, sequencing of all mtDNA-encoded tRNAs did not reveal any mutations. These patients also may have a mutation in either the mtDNA-encoded ribosomal RNA genes or more likely, a mutation in a nDNA gene encoding proteins required for mitochondrial translation. One patient had a heteroplasmic T8993G mutation, which is common cause of LS.

Although a limited study based in a single tertiary medical center, our results suggest that isolated complex I defects may be the most frequent cause of LS in Korea and more common than previously reported [8,9]. An increasing number of papers have described mutations in nuclear encoded complex I subunits [10-12]; however, in our series, we identified mutations of mtDNA encoded *ND* genes in 4 (44%) out of 9 patients with isolated complex I deficiency. This remarkable result indicates that, in contrast to previous reports [13], mtDNA mutations may be responsible for a significant proportion of the infants and children with complex I defects, and targeted mtDNA screening based on biochemical results is an essential step in the diagnostic evaluation of pediatric mitochondrial disorders.

A major cause of mitochondrial disease in infancy and childhood, isolated complex I deficiency manifests a wide clinical spectrum ranging from severe encephalopathy to isolated hypertrophic cardiomyopathy [12,14]. Mutations in 16 mtDNA and nuclear genes have accounted for less than half of all patients with complex I deficiency [15,16], so many other causative genes are likely to be identified.

Although persistently elevated plasma lactate or characteristic histological changes of mitochondria in muscle are typical features of mitochondrial diseases, in our LS patients, half had normal plasma lactate and the majority had normal muscle morphology. Therefore, the usual screening tests for mitochondrial disease are not very sensitive in patients with LS, particularly when due to mtDNA mutations in protein coding genes [17,18]. Brain magnetic resonance spectroscopy (MRS) with voxels on brain lesions can reveal elevated lactate, which can be an important clue to the diagnosis of LS [19].

In this study, biochemical or molecular defects were identified in 14 patients (87.5%). We identified mtDNA mutations in one-third of the patients. In the remaining two-thirds, who are likely to have mutations in nDNA-encoded subunits of respiratory chain enzymes, we could not identify genetic causes. Identifying the genetic etiology of Leigh syndrome in Korean patients remains a persistent challenge for clinicians.

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Table 1
Clinical, biochemical and molecular genetic characteristics in 16 patients

Patient	Onset age/sex	Initial Sx	Lactate	Pathology	Biochemistry	Mol. Genetics
1	1 yr/F	Dev. reg.	Normal	RRF(-)	I + III + IV	NI
2*	6 yr/F	Dystonia	Normal	RRF(-), SSVs(+)	I	G10197A(ND3)
3*	4 yr/M	Dystonia	Normal	RRF(-)	I	G10197A(ND3)
4†	6 mo/M	Dev. reg.	Normal	RRF(-)	Normal	NI
5†	10 mo/F	Dev. reg.	Normal	RRF(-)	Normal	NI
6	6 mo/M	Stroke	High	RRF(-)	I	G10197A(ND3)
7	5 mo/F	Dev. reg.	High	RRF(-)	I	NI
8	6 mo/F	IS	High	RRF(-)	I	NI
9	2 mo/F	Dev. reg.	High	RRF(-)	I	NI
10	6 mo/F	Dev. reg.	High	RRF(-)	I + III + IV	NI
11	6 mo/F	Dev. reg.	Normal	RRF(-)	I	NI
12	3 mo/F	Spasms	Normal	RRF(-)	I + III + IV	NI
13	2 mo/M	IS	Normal	RRF(-)	I	NI
14	9 mo/M	Dev. reg.	High	RRF(-)	I + III + IV	NI
15	1 yr/M	Ataxia/Szs	High	RRF(-), SSVs(+)	I	G13513A(ND5)
16	2 yr/F	Dev. reg.	High	RRF(-)	Not done	T8993G

Dev. reg., developmental regression; IS, infantile spasms; Szs, seizures; SSVs, strong succinate dehydrogenase positive vessels; NI, not identified.

* are siblings with same clinical manifestations

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