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Genotype-phenotype correlations of the mitochondrial DNA mutations in Leigh syndrome

Ji-Hoon Na

Department of Medicine
The Graduate School, Yonsei University

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연세대학교
YONSEI UNIVERSITY

Genotype-phenotype correlations of the mitochondrial DNA mutations in Leigh syndrome

Directed by Professor Young-Mock Lee

The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Doctor of Philosophy

Ji-Hoon Na

June 2019

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This certifies that the Doctoral Dissertation of Ji-Hoon Na is approved.

Thesis Supervisor : Young-Mock Lee

Thesis Committee Menber#1 : Se Hoon Kim

Thesis Committee Menber#2 : Joon Won Kang

Thesis Committee Menber#3 : Lark Kyun Kim

Thesis Committee Menber#4 : Soon Min Lee

The Graduate School

Yonsei University

June 2019

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<TABLE OF CONTENTS>

연세대학교
YONSEI UNIVERSITY

연세대학교
YONSEI UNIVERSITY

LIST OF FIGURES

연세대학교
YONSEI UNIVERSITY

LIST OF TABLES

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ABSTRACT

Genotype-phenotype correlations of the mitochondrial DNA mutations in Leigh syndrome

Ji-Hoon Na

Department of Medicine

The Graduate School, Yonsei University

(Directed by Professor Young-Mock Lee)

Leigh syndrome is the most common clinical presentation of mitochondrial diseases in children and is genetically divided into mitochondrial DNA (mtDNA)-associated Leigh syndrome and nuclear gene-encoded Leigh syndrome, both of which differ in genotypes, prevalence and clinical features. The mtDNA-associated Leigh syndrome is influenced not only by the pathogenicity of the mutant gene itself, but also by the relative amounts of mutant and wild-type mtDNA, heteroplasmic mutant load. We aim to investigate the genotype-phenotype correlations by a quantitative analysis of heteroplasmic mutant load and subgrouping of genotypes in genetically confirmed patients with mtDNA-associated Leigh syndrome.

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We performed whole mitochondrial DNA sequencing with next generation sequencing (NGS) technology on 130 patients clinically diagnosed with Leigh syndrome in a single tertiary medical institution in Korea. As a result, mtDNA mutations were detected in 31 patients with clinical Leigh syndrome. However, four of them were excluded from this study because they had currently unverified variants in the academic literature. Finally, 27 patients with Leigh syndrome were identified as having Leigh syndrome with mtDNA mutation. The patients with genetically confirmed Leigh syndrome with mtDNA mutations were additionally tested for mutant load by NGS. We reviewed the clinical manifestations of the patients included in this study with reference to stringent diagnostic criteria for clinical diagnosis of Leigh syndrome. Laboratory findings, brain imaging (MRI, MRS) and data from long-term follow-ups are investigated for phenotype analysis. In order to investigate the relationship between genotypes and various phenotypes around mutant loads, we tried to understand patterns and relationships through scatter plots and bar-plots for data visualization.

The first symptom of the patients was developmental delay or declining, which accounted for the majority of the whole (48.1%), followed by seizure (11.1%), ataxia (11.1%), hypotonia (11.1%), visual disturbance (11.1%) and gait disturbance (7.4%). Basal ganglia involvement was found in all patients regardless of the pathologic genotype or nucleotide change, and high involvement was observed in the brain stem (N = 18, 66.7%), followed by

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involvement of the thalamus (N = 14, 51.9%), cerebral atrophy (N = 14, 51.9%) and cerebellar atrophy (N = 12, 44.4%). The median year from diagnosis of Leigh syndrome to last follow-up was 5.4 years (ranged from 0.1 to 12.1 years). Nine patients received respiratory support (33.3%) and 10 patients deteriorated with oxygen dependence (37.0%). Nine patients (33.3%) were assisted by enteral tube feeding.

After whole mtDNA sequencing, gene confirmed mtDNA Leigh syndrome could be classified as 6 genotypes or 10 mtDNA nucleotide changes. The range of mutant load of the patients ranged from 55.5 to 100%, and the median value was 96.3%. The difference of distribution of the mutant load of these three major genes was statistically significant. *MT-ATP6* showed a relatively high mutant load, while *MT-ND5* had a relatively low mutant load. Compared with other genotypes, the *MT-ATP6* gene has a significantly higher amount of mutant load, a wider organ involvement status, and a greater number of first symptoms. In particular, *MT-ND3* is thought to have a very strong association with epilepsy, and related pathologic nucleotide changes are noted as m.10191T>C. The need for supportive equipment, such as respiratory supports, oxygen dependency and enteral tubes, also were greater in *MT-ND3* than other genotypes. In patients with a mutation of the *MT-ND5* gene (m.13513G> A), a characteristic cardiologic involvement of 75% was observed (N = 3), which is in contrast to the absence of cardiac involvement in *MT-ATP6*.

In conclusion, we found features of mtDNA Leigh syndrome that were

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not found in several previous studies. First, the distributions of heteroplasmic mutant loads vary according to the gene, so if we collect enough patients for the same gene or the same nucleotide change, we can conclude a more specific and general conclusion. Second, pathologic variants of mitochondrial genotypes and their associated pathologic nucleotide changes have newly been found to be associated with some phenotypes of mtDNA associated with Leigh syndrome. Even with the difficulty of statistical analysis, this is a further step forward than the previous genotype-phenotype correlation of existing qualitative analysis, and this study would ultimately provide a good basis for interpretation of clinical results, genetic counseling and genetic targeted therapy of Leigh syndrome.

Keywords: leigh syndrome, mtDNA, genotype, phenotype, heteroplasmy, mitochondrial diseases, mutant load

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Genotype-phenotype correlations of the mitochondrial DNA mutations in Leigh syndrome.

Ji-Hoon Na

Department of Medicine

The Graduate School, Yonsei University

(Directed by Professor Young-Mock Lee)

I. INTRODUCTION

Mitochondria plays a key role in providing biochemical energy to cells by producing adenosine-5'-triphosphate (ATP) through oxidation of nutrients known as oxidative phosphorylation (OXPHOS).[1-3] Mitochondrial diseases is a large heterogeneous group of disorders that is caused by the primary dysfunction of the mitochondrial respiratory chain. This disease is characterized by multi-system dysfunction, a wide spectrum of clinical symptoms and generally characterized by poor genotype-phenotype correlation.[2] When a mitochondrial dysfunction occurs, it is a problem at the cellular level, so a very diverse and complex syndrome called ‘mitochondrial disease’ appears.[3]

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Mitochondrial diseases are rare diseases which have a prevalence of approximately 1 in 10,000 people as a whole. But the prevalence of carriers is known to be 1 in 200 people.[2] From a genetic point of view, there are two genetic approaches to mitochondrial diseases, mitochondrial DNA (mtDNA) and nuclear DNA (nDNA).[4-6] Moreover, due to maternal inheritance, heteroplasmy and the threshold effect of mtDNA, the same tissues containing a different mtDNA mutation and different tissues containing the same mtDNA mutation may be affected to variant degrees.[1,2] These aspects contribute to the extreme genotype and phenotypic diversity of the mitochondrial diseases, affecting the difference in severity of the diseases and various clinical outcomes of multiple organs, and consequently the various clinical departments that must be involved in the treatment and management of the diseases.[5,6]

Among the mitochondrial diseases, Leigh syndrome (or subacute necrotizing encephalomyelopathy) is the most common clinical presentation of mitochondrial diseases in children, with a prevalence of approximately 40,000 newborns.[7,8] Leigh syndrome was first introduced by Denis Leigh in 1951 and is characterized by onset of symptoms typically between 3 ~ 12 months, often following a viral infection.[7-9] Psychomotor retardation, progressive neurologic decline, extensive neurologic features (e.g. hypotonia, spasticity, dystonia, muscle weakness, hypo- or hyperreflexia, seizures, infantile spasms, movement disorders, cerebellar ataxia, peripheral neuropathy) and extra-neurologic manifestations (e.g. hypertrophic cardiomyopathy, conduction anomaly,

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ophthalmological manifestations, renal tubulopathy) may appear as symptoms of Leigh syndrome.[10-16] Approximately 50% of patients diagnosed with this syndrome die by age 3, most of whom die from respiratory or heart failure.[9,17]

Leigh syndrome can be suspected based on clinical features, laboratory findings (e.g. lactate elevation in blood and/or cerebrospinal fluid), brain imaging, histopathology of muscle tissue and biochemical analysis. In addition, recent advances in molecular genetic testing approaches, such as targeted single-gene testing, mitochondrial genome sequencing, whole exome sequencing (WES) and more comprehensive genomic testing, have led to a rapid increase in genetic understanding of Leigh syndrome.[9,17-22] Leigh syndrome is genetically divided into mitochondrial DNA (mtDNA)-associated Leigh syndrome and nuclear gene-encoded Leigh syndrome, both of which differ in genotypes, prevalence, clinical features and treatment options. More than 75 genes have been identified to date for Leigh syndrome, of which 14 are known to be mtDNA-associated Leigh syndrome (e.g. *MT-ATP6, MT-CO3, MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND5, MT-ND6, MT-T1, MT-TK, MT-TL1, MT-TL2, MT-TV, MT-TW*). The mtDNA-associated with Leigh syndrome are transmitted by maternal inheritance, and clinical manifestations of mtDNA pathogenic mutations are influenced not only by the pathogenicity of the mutant itself, but also by the relative amounts of mutant and wild-type mtDNA, heteroplasmic mutant loads.[9,16,17]

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Although genotype-phenotype correlations are difficult to elucidate due to the diversity of these mtDNA pathogenic mutations, these studies are essential for understanding the effects of genetic variants on phenotype in Leigh syndrome, and through this process, we can come to the precise treatments of the genetic basis. However, since Leigh syndrome is a rare disease, it is difficult to make a large cohort to study. Therefore, there is little research related to genotype-phenotype correlation in Leigh syndrome.[23] Recently, Kalliopi Sofou et al. presented a remarkable study of genotype-phenotype correlation in Leigh syndrome. In this study, they provided new insights into the phenotypic panorama of certain genotypes of Leigh syndrome.[24]

However, their study did not consider the relationship between heteroplasmic mutant loads according to the subgroup of each genotype and phenotypes. Since mtDNA-associated Leigh syndrome exhibits a variety of clinical manifestations depending on the heterogenous mutant loads,[17] studies investigating the relationship between heterogenous mutant loads and phenotypes may be a deeper approach to understanding the genotype-phenotype correlation of Leigh syndrome. Over the past several decades, some research and analyses attempted to show the relevance of heterogenous mutant load and clinical phenotypes in certain genotypes in Leigh syndrome.[25-30] However, the results of these studies have not shown clear relationships between mutant load and clinical manifestation due to limitations of diagnostic

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modalities. But now, with the development of genetic and other diagnostic techniques, we can expect more advanced results in this research.

In this study, we aimed to investigate the expression pattern of Leigh syndrome phenotypes with a quantitative analysis of heteroplasmic mutant loads according to subgroup of genotype. Therefore, we sought to explore the relationship between phenotype, gene mutation, and heteroplasmic mutant load in mtDNA associated Leigh syndrome in a single tertiary medical institution in the Republic of Korea.

II. MATERIALS AND METHODS

1. Selection of patients (Figure 1)

Among patients whose clinical phenotypes were suspected to be Leigh syndrome in the Mitochondrial Disease Clinic of Gangnam Severance Hospital, the Republic of Korea, we selected 130 patients with clinically diagnosed Leigh syndrome. The clinical diagnosis of Leigh syndrome was based on stringent diagnostic criteria for Leigh syndrome by Rahman et al.[8,9] The criteria is outlined as follows: a progressive neurologic disease with motor and intellectual developmental delays, signs and symptoms of brain stem and/or basal ganglia disease, raised lactate concentration in blood and/or cerebrospinal fluid (CSF), bilateral symmetric hyperintense signal abnormality in the brain stem and/or basal ganglia on T2-weighted image in brain magnetic resonance

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imaging (MRI), etc. Sequencing of whole mitochondrial DNA was performed in all patients through NGS technology. Among them, patients with mtDNA mutations were detected. And then, an extensive literature search was performed to confirm whether the mtDNA mutation of each patient was currently reported, and patients with mtDNA variants whose pathogenicity were not confirmed so far, were excluded from this study. Finally, we only included patients with mtDNA mutations identified as pathogenic variants in this study. As a result, mtDNA mutations were detected in 31 patients with clinical Leigh syndrome. However, four of them were excluded from this study because they were currently unverified variants reported in the academic literature. Finally, 27 patients with Leigh syndrome were identified as having Leigh syndrome with mtDNA mutation. In addition, the patients who were genetically confirmed as having Leigh syndrome with mtDNA mutations were additionally tested for mutant load.

Every parent received a detailed explanation and signed their informed consent before participating in the study. This study was approved by the Institutional Review Board of Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, the Republic of Korea following the tenets of the Helsinki Declaration.

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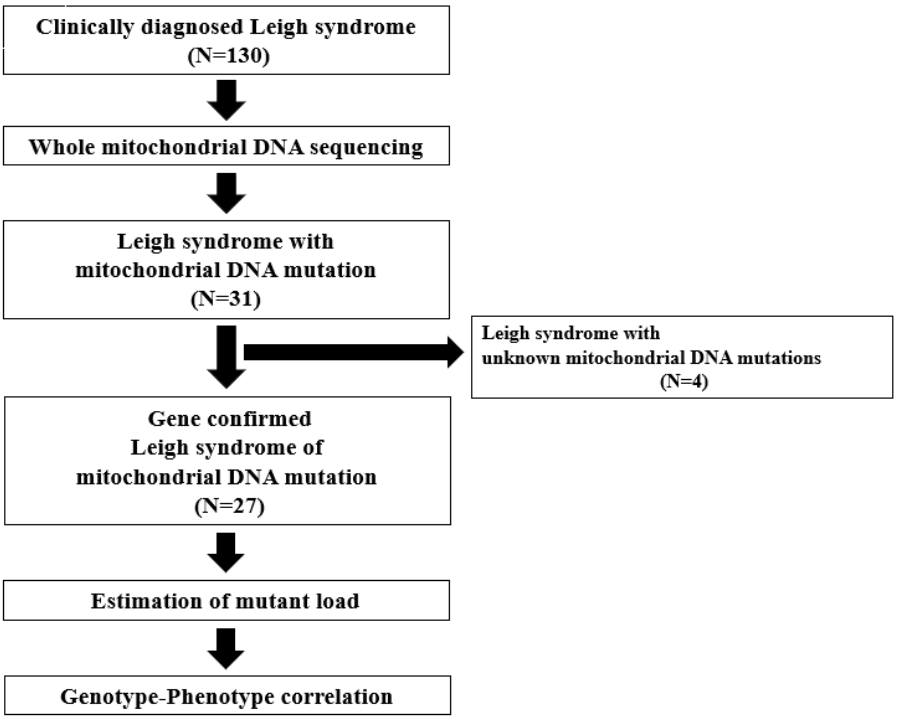

Figure.1 Study design and Selection of patients

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2. Phenotype analysis

(1) Clinical manifestations

We reviewed the clinical manifestations of Leigh syndrome patients included in this study with reference to stringent diagnostic criteria for clinical diagnosis of Leigh syndrome.[8,9] The first symptom at onset of the disease, organ involvement at the time of last follow-up, age of diagnosis as Leigh syndrome and time interval from age of first symptom to age of diagnosis as Leigh syndrome were investigated as general characteristics of the patients. Patients were divided into two groups according to the criteria of early onset Leigh syndrome: age of the first symptom was less than 24 months and more than 24 months. In the first symptom at onset of the disease, we identified the most dominant symptom of the patients. In organ involvement at the time of last follow-up, we checked all the organs involved with Leigh syndrome at that time. Therefore, the number of patients involved in each organ was counted.

(2) Laboratory findings

Results of plasma lactate level and plasma lactate/pyruvate ratio of the patients were obtained, and the degree of plasma lactate level is defined as mild, moderate or severe if the increase above the normal reference value is at least 2-fold, 3-fold, or 4-fold, respectively.[6,36] They were treated as 1 to 4 sequential variables. It is known that a lactate/pyruvate (L/P) ratio over 20 in blood is an

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indication of dysfunction of the respiratory chain, and this was also applied in this study.[47] We explored the relationship between these results and type of mtDNA mutations or mutant loads of mtDNA. We also performed muscle biopsy and observed light microscopic and electron microscopic changes of obtained muscles of the patients. Light microscopy revealed the presence of specific findings for mitochondrial diseases such as ragged red fiber, and electron microscopy revealed pleoconia or megaconia. The heteroplasmic mutant load was measured quantitatively as a step in the whole mitochondrial DNA sequencing process for enrolled Leigh syndrome patients.

(3) Brain MRI and proton magnetic resonance spectroscopy (MRS) findings

mtDNA-associated Leigh syndrome is characterized by bilateral symmetric hyperintense signal abnormality in the brain stem and/or basal ganglia on T2-weighted MRI findings.[17] We obtained MRI findings of patients and divided them according to the affected area, such as the basal ganglia, brain stem, thalamus and other parts of the brain. It is known that MRS can also be useful in detecting lactate peaks in the brain.[48] Therefore, MRS abnormalities were obtained to establish the diagnosis.

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(4) Long-term follow-up

We prospectively identified prognostic indicators with long-term follow-up of enrolled patients. Time interval from diagnosis of Leigh syndrome to last follow-up was calculated. In addition, we investigated the factors important for supportive care of Leigh syndrome, such as respiratory support, oxygen dependency and enteral tube feeding. Also, the current progression of Leigh syndrome patients was graded into three stages as 'mild - ambulatory and/or independent for daily activities', 'moderate - partially dependent for daily activities, able to express and understand direction' and 'severe - bedridden, total dependency for daily activities[6].

3. Genotype analysis

(1) Whole mitochondrial gene sequence analysis by NGS technology

In the mitochondrial genome, pathogenic mutations, including point mutations and large deletions, can occur throughout the entire genome. Therefore, diagnosis of mtDNA-related Leigh syndrome should include the detection and quantification of sequence changes at any position of the mitochondrial genome.[20] Whole mitochondrial gene sequence analysis was performed in all patients who were clinically diagnosed with Leigh syndrome in this study. The NGS technology was used to confirm mtDNA Leigh

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syndrome genetically and quantify heteroplasmic mutant load of mtDNA. The sequence results were compared with the human mitochondria reference (GenBank ID: NC_012920.1).[20, 31-32] Several mtDNA mutations were detected in enrolled patients, and they were sub-grouped by the same mutation (e.g. *MT-ATP6, MT-ND1, MT-ND3, MT-ND4, MT-ND5, MT-ND6*).

(2) Sample preparation

DNA was extracted from peripheral blood leukocytes or primary cultured fibroblast cells with the QIAcube System and QIAamp DNA Blood Mini Extraction Kit (Qiagen, California) and were stored in 10mM Tris buffer solution at –20°C. MtDNA was amplified using long range PCR. PCR reaction conditions were 98°C for 30 seconds, 30 cycles of 98°C for 10 seconds, 72°C for 8 minutes 15 seconds with a final extension of 72°C for 10 minutes. PCR products were run on a 1% agarose gel, then the expected 16.5 Kb fragments were excised. DNA was purified using Agencourt AMPure XP (Beckman Coulter). Quantification was assessed by 4200 TapeStation (Agilent, UK).[39]

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(3) Library preparation and sequencing

We fragmented the PCR product into 150 to 200 basepair (bp) segments with a NEBNext dsDNA Fragmentase® (New England Biolabs, UK), according to the manufacturer's protocol. The enzyme fragmented PCR product was used as input to the Accel-NGS® 2S PCR-free DNA Library Kit following the manufacturer's protocol. Final libraries were evaluated on the 4200 TapeStation (Agilent, UK) and quantitated by Qubit (Thermo Fisher Scientific). Libraries were sequenced by synthesis on Miseq for paired 150 bp read lengths using Illumina MiSeq V3 Kits (Illumina, USA).[39-41]

(4) Analysis of sequence and detection of variants

The sequenced reads were mapped to the human mitochondria reference (NC_012920) with Burrows-Wheeler Aligner (BWA), and variants were identified with the Genome Analysis toolkit (GATK). Sequence variants were filtered according to various quality parameters. In NGS technology, each template is sequenced individually, so quantitative analysis of heteroplasmic mutant load is possible by counting the number of mtDNA reads [42,43]

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4. Data analysis and statistical methods

Statistical analysis was performed mainly with R for Windows version 3.5.2 (http://cran.r-project.org) and SPSS version 20.0 for Windows (IBM Corp., Armonk, NY, USA). Descriptive statistics were used including the median and range. Chi-square tests, parametric t-tests and one-way analysis of variance (ANOVA) test followed by post hoc analysis were used to evaluate differences between the groups when discussing general characteristics. Correlation tests were also performed to determine the association between variables. The mutation load of mtDNA in each patient was displayed as a percentage, and it was treated as a continuous variable in the statistical analysis. The mutation loads of each mtDNA Leigh syndrome genotype were compared with various phenotypes.

In order to investigate the relationship between gene types and various clinical variables around mutant loads, we tried to understand the patterns and relationships through scatter plots and bar-plots for data visualization. Despite their simplicity, scatter plots are widely used as a powerful tool for visualizing multifactorial and complicated data. In this study, visualizing analysis using scatter plots was actively used to determine the relationship among various phenotypes, sub-grouped genotypes and heteroplasmic mutant loads of Leigh syndrome with mtDNA mutations.

P-values less than 0.05 will be considered statistically significant.

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However, even if the p-value is slightly over 0.05, it might be considered clinically meaningful, the analysis will be described with a cautious view.

III. RESULTS

1. Phenotypic analysis

(1) General characteristic of the mtDNA gene confirmed 27 patients (Table 1)

In all 130 patients clinically diagnosed with Leigh syndrome who underwent whole mitochondrial DNA sequencing, mitochondrial DNA mutations were found in 31 patients. Of these, except for 4 patients with unknown mutations, there were 27 cases of genetically confirmed mtDNA Leigh syndrome. Nine patients (33.3%) were male. The first symptom of most patients (20, patients, 74.1%) occurred before 24 months, consistent with early onset Leigh syndrome criteria.

The first symptom of the patients was developmental delay or declining, which accounted for the majority of the whole (48.1%), followed by seizure (11.1%), ataxia (11.1%), hypotonia (11.1%), visual disturbance (11.1%) and gait disturbance (7.4%). The organ involvement was investigated at the time of the last visit to the patient's hospital. As a result, the central nervous

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system was involved in all patients, and the eye (59.3%), respiratory system (44.4%) and gastrointestinal tract (40.7%) also had a relatively high frequency. The median age at which patients were diagnosed with Leigh syndrome was 28 months, ranging from 5 months to 244 months. The median age of the time interval from age of first symptom to age of Leigh syndrome diagnosis is 9 months, ranged from 1 month to 136 months.

Table 1. General characteristics of the patients

	Total (N=27)
Gender (male, %)	9 (33.3)
Age of first symptom (months, range)	15 (0 ~ 119)
<24mo	20 (74.1)
24mo ~ 60mo	5 (18.5)
> 60mo	2 (7.4)
First symptom at onset of LS, n (%)	
Developmental delay or declining	13 (48.1)
Seizure	3 (11.1)
Gait disturbance	2 (7.4)
Ataxia	3 (11.1)
Visual disturbance	3 (11.1)
Hypotonia	3 (11.1)
Organ involvement, n (%)	
Central nervous system	27 (100)
Muscular system	9 (33.3)
Eye	16 (59.3)
Respiratory system	12 (44.4)
Heart	6 (22.2)
Gastrointestinal tract	11 (40.7)
Endocrine system	8 (29.6)
Ear	3 (11.1)
Kidney	8 (29.6)
Skeletal	3 (11.1)
Age at the time of diagnosis of LS (months, range)	28 (5~244)
Time interval from age of first symptom to age of LS diagnosis (month, median, range)	9 (1~136)

Abbreviation: LS; Leigh syndrome

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(2) Mitochondrial characteristic of the mt-DNA gene confirmed 27 patients (Table 2)

The characteristics related to mitochondrial disease in all patients were investigated. Serum lactic acidosis was obtained at the time of diagnosis. They were graded as 'mildly increased' over 2-fold, 'moderately increased' over 3-fold, and 'severely increased' over 4-fold, based on the normal margin of the upper margin. A muscle biopsy was performed on 25 patients, excluding 2 in the total number of patients. As a result, specific findings for mitochondrial diseases such as light microscopic changes (ragged red fibers), were found in 5 patients (20%). Abnormal findings (pleoconia or megaconia or both) on electron microscopic changes were found in 9 patients (36%).

연세대학교
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Table 2. Mitochondrial characteristics of the patients

Mitochondrial variables	**Total (N=27)**
Serum lactic acidosis at diagnosis (mmol/L)	3.1 (1.2 ~ 6.8)
Grading of serum lactic acidosis, n (%)	
Normal	3 (11.1)
Mildly increased (≥ 2-fold)	12 (44.4)
Moderately increased (≥ 3-fold)	9 (33.3)
Severely increased (≥ 4-fold)	3 (11.1)
Muscle biopsy obtained, n (%)	**Total (N=25)**
Light microscopic changes	
Specific findings for mitochondrial diseases	5 (20.0)
Nonspecific findings	2 (8.0)
Normal	18 (72.0)
Electron microscopic changes	
Pleoconia only	2 (8.0)
Megaconia only	0
Pleoconia and Megaconia	7 (28.0)
Normal	16 (64.0)

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(3) Relationship of mutant load and MRI/MRS involvement (Table 3)

Pathologic variants of mitochondrial genotypes and mutations associated with pathologic nucleotide changes were investigated. There was no clear correlation between mutant load and MRI involvement patterns. Basal ganglia involvement was found in all patients regardless of the pathologic genotype or nucleotide change, and high involvement was observed in the brain stem (n = 18, 66.7%), followed by involvement of the thalamus (n = 14, 51.9%), cerebellar atrophy (n = 14, 51.9%) and cerebellar atrophy (n = 12, 44.4%). MRS was performed in 25 patients. As a result, lactate peak was observed in 18 patients (66.7%).

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Table 3. MRI/MRS involvement of the patients

MRI/MRS findings	Total (N=27)
Magnetic resonance imaging obtained, n (%)	
Basal ganglia	27 (100)
Thalamus	14 (51.9)
Brain stem	18 (66.7)
Midbrain	18 (66.7)
Pons	9 (33.3)
Medulla	13 (48.1)
Cerebral atrophy	14 (51.9)
Cerebellar atrophy	12 (44.4)
Cortex/subcortex signal abnormality	8 (29.6)
White matter signal abnormality	6 (22.2)
Magnetic resonance spectroscopy obtained, n (%, N = 25)	N=25
Presence of lactate peak	18 (66.7)
Decreased NAA peak	10 (37.0)
Normal	3 (11.1)

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(4) Follow-up characteristics of the mtDNA genes confirmed 27 patients (Table 4)

Clinical status of each patient was assessed based on the last follow-up date. The median year of diagnosis of Leigh syndrome to last follow-up was 5.4 years (ranged from 0.1 to 12.1 years). Nine patients received respiratory support (33.3%) and 10 patients deteriorated with oxygen dependence (37.0%). Nine patients (33.3%) were assisted by enteral tube feeding. Finally, all of patients were classified into three levels of clinical severity: 'mild': ambulatory and / or independent for daily activities, 'moderate' wheelchair bound and / or partially dependent for daily activities and 'severe': bedridden and totally dependent for daily activities. Most patients (n = 16, 59.3%) showed moderate clinical severity at the last follow-up.

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Table 4. Follow-up characteristics of the patients

	Total (N=27)
Time from diagnosis of LS to Last F/U (year, median, range)	5.4 (0.1 ~ 12.1)
Respiratory support	9 (33.3)
O_2 dependency	10 (37.0)
Enteral tube feeding	9 (33.3)
Clinical severity	n (%)
1. Mild (ambulatory and/or independent for daily activities)	7 (25.9)
2. Moderate (wheel chair bound and/or partially dependent for daily activities)	16 (59.3)
3. Severe (bedridden and total dependent for daily activities)	4 (14.8)

Abbreviation: LS; Leigh syndrome

2. Genotypic analysis (using NGS technology)

(1) Genotype and nucleotide change of all patients (Table 5, Figure 2,3)

In our study, gene confirmed mtDNA Leigh syndrome could be classified with 6 genotypes or 10 mtDNA nucleotide changes. The genotype of each mitochondrial DNA nucleotide change was classified based on the reference that pathologic variants were confirmed up to date. As a result, six genotypes could be classified into 27 patients. Among them, *MT-ATP6* was the most common in 13 patients, followed by *MT-ND3* (n = 7) and *NT-ND5* (n = 4). *MT-ND1, MT-ND4*, and *MT-ND6* were only found in one patient each, so a comparative analysis was not possible. In the aspect of the mtDNA nucleotide changes, m.8993T>G (n=5), m.8993T>C (n=4), m.10191T>C (n=6), m.13513G>A (n=4) and m.9176T>C (n=3) were relatively large, accounting

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for 81.5% of the total patients.

Table 5. Classification of genotype corresponding to Mitochondrial DNA Nucleotide change

Genotype	Mitochondrial DNA Nucleotide change	Cases (n)
MT-ATP6 (n=13)	m.8993T>G	5
	m.8993T>C	4
	m.9176T>C	3
	m.9185T>C	1
MT-ND1 (n=1)	m.3697G>A	1
MT-ND3 (n=7)	m.10191T>C	6
	m.10158T>C	1
MT-ND4 (n=1)	m.11777C>A	1
MT-ND5 (n=4)	m.13513G>A	4
MT-ND6 (n=1)	m.14459G>A	1

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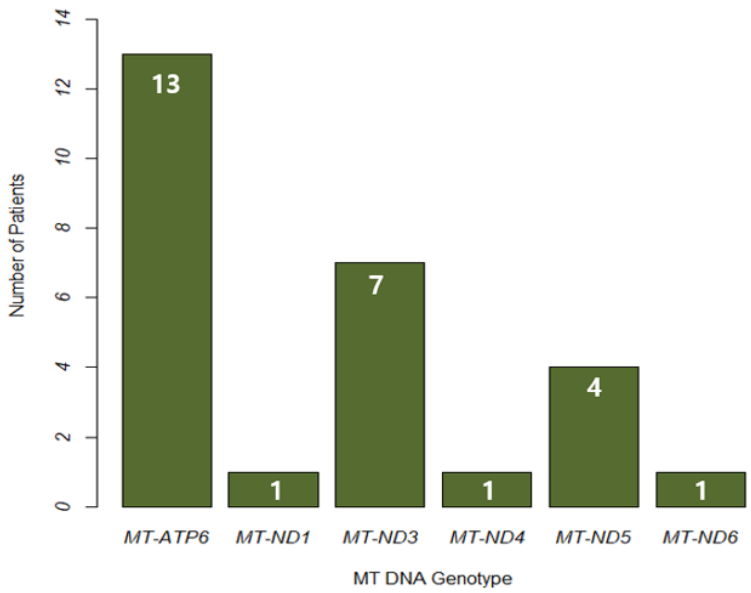

Figure 2. Distribution of all patients by genotype of mitochondrial DNA associated Leigh syndrome

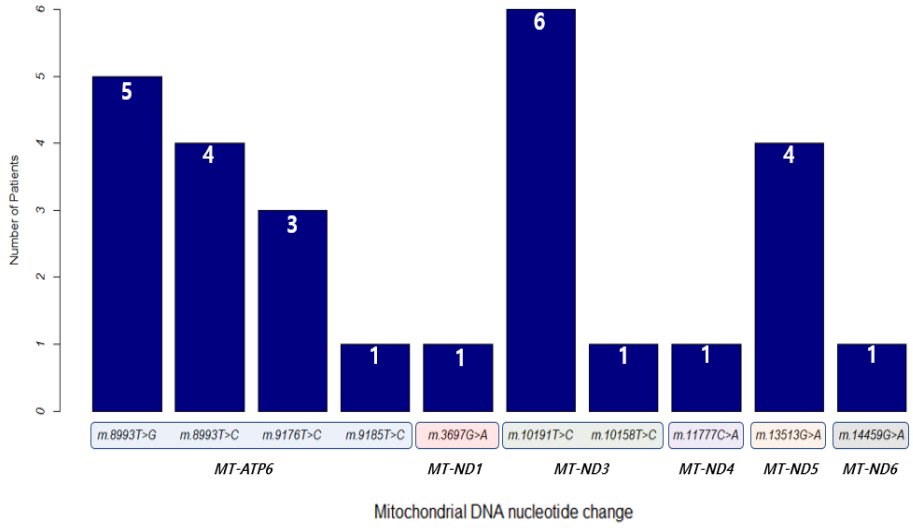

Figure 3. Distribution of all patients by mitochondrial DNA nucleotide change

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(2) Heteroplasmic mutant load of the patients (Figure 4,5,6)

The range of mutant load of the patients ranged from 55.5 to 100%, and the median value was 96.3%. The difference of distribution of the mutant load of the three major genes (*MT-ATP6, MT-ND3, MT-ND5*) was statistically significant. *MT-ATP6* showed a relatively high mutant load, while *MT-ND5* had a relatively low mutant load. We investigated the distribution of mutant load by nucleotide change. The m.8993T>G, m.8993T>C, m.9176T>C and m.9185T>C which belong to *MT-ATP6* showed a very high distribution of mutant load of more than 90%. The median value of mutant load of m.10191T>C, which occupies most of *MT-ND3*, was about 80%, and the median value of mutant load of m.13513G>A, which is the nucleotide change of mutation of *MT-ND5*, was less than 70%. The correlation study between mutant load and age of first symptom was not statistically significant (p = 0.210). Also, the correlation between mutants load and age of diagnosed with Leigh syndrome was not statistically significant (p = 0.215).

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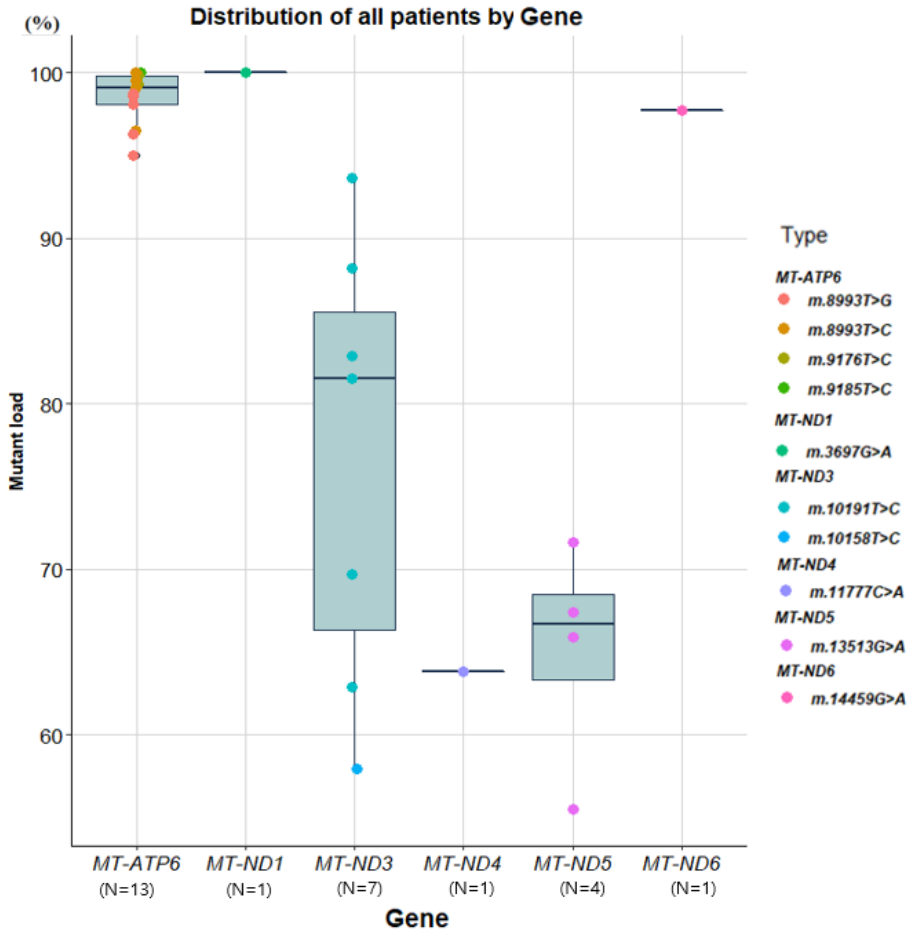

Figure 4. Distribution of mutant load in all patients by gene

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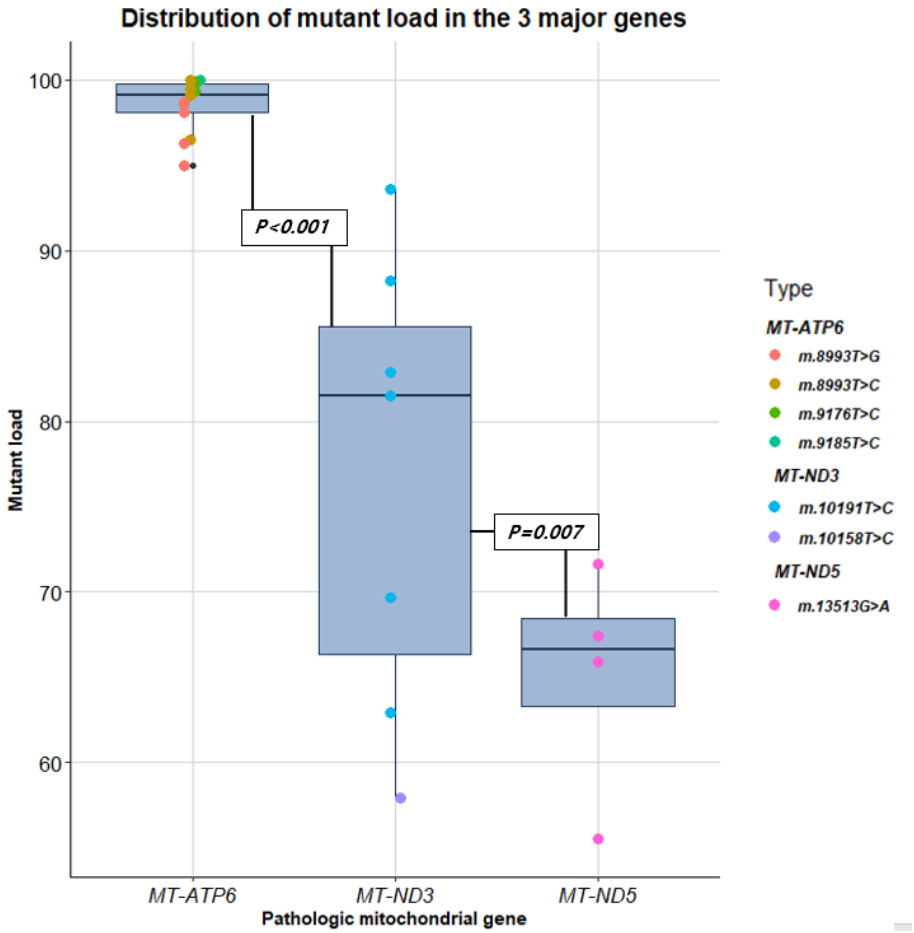

Figure 5. Distribution of mutant load in the 3 major genes.

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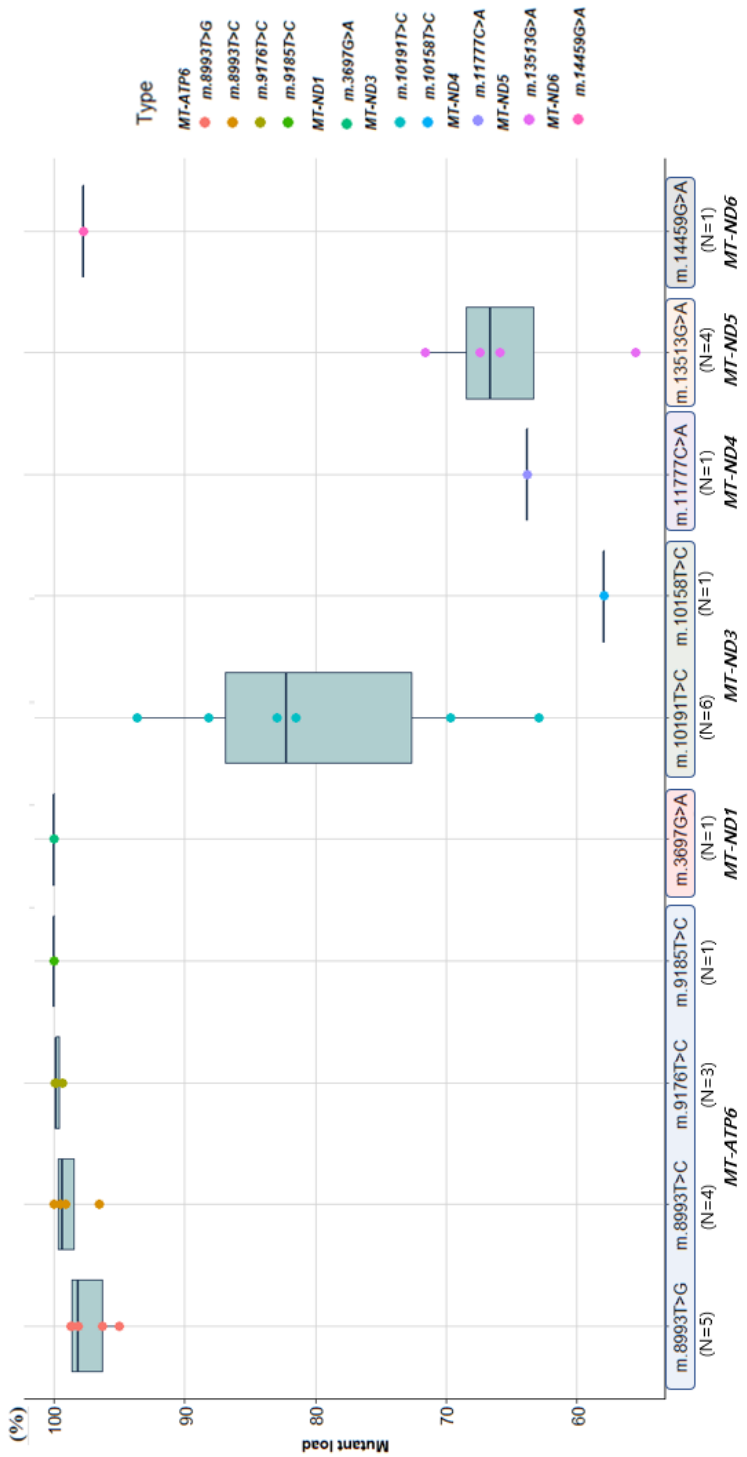

Figure 6. Distribution of mutant load in all patients by nucleotide change

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3. Genotype-phenotype correlation

(1) Distribution of the patients' mutant load and age of first symptom (Figure 7)

The distribution of patients according to mutant load and age of first symptom was examined. There were 15 patients (55.6%) with more than 80% of mutant load and less than 24 months of onset of symptoms. The mt-DNA mutant genotypes corresponding to this part were *MT-ATP6* and *MT-ND1*, in which nucleotide changes are m.8993T> G, m.8993T> C and m.9176T> C. The distribution of the first symptom age of the *MT-ATP6* mutations in the patients varied but was limited to 24 months. The mtDNA mutant load showed relatively high levels in patients with the *MT-ATP6* mutation, while the *MT-ND5* mutation of patients showed relatively low mutant load. We have conducted a correlation study on the relationship between mtDNA mutant load and age of first symptom. However, there was no significant correlation between mutant load and age of first symptom at the time of diagnosis ($p = 0.210$).

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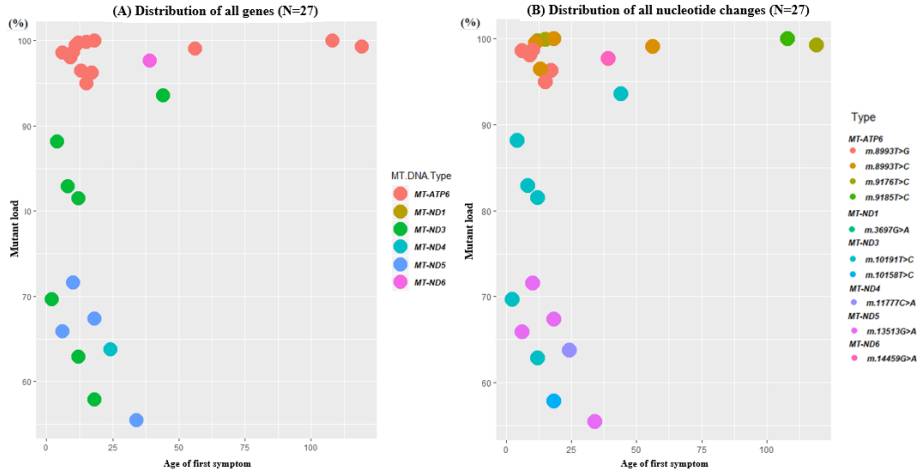

Figure 7. Scatter plot of relationship of mutant load and age of first symptom.

(A) In the aspect of pathologic variants of mitochondrial genotypes.

(B) In the aspect of pathologic nucleotide changes.

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(2) Relationship of the mtDNA mutant load and first symptom (Figure 8)

Following the correlation and distribution study of mutant load and first symptom age, we examined the relationships of mutant load and first symptom. The first symptom of all patients could be divided into 6 categories: developmental delay (DD) and regression, gait disturbance, seizure, hypotonia, visual disturbance and ataxia. The relationship between the first symptom and the mutant load was not observed statistically as a whole, but some locally remarkable features were observed. First, DD and regression were the first symptom (48.4%) in most patients regardless of *MT-DNA* mutation genotype and mutant load. Second, the mutant load of patients who experienced gait disturbance as first symptom was generally very high. Third, all patients who experienced seizure as the first symptom had *MT-ND3* (nucleotide change m.10191T> C) mutations.

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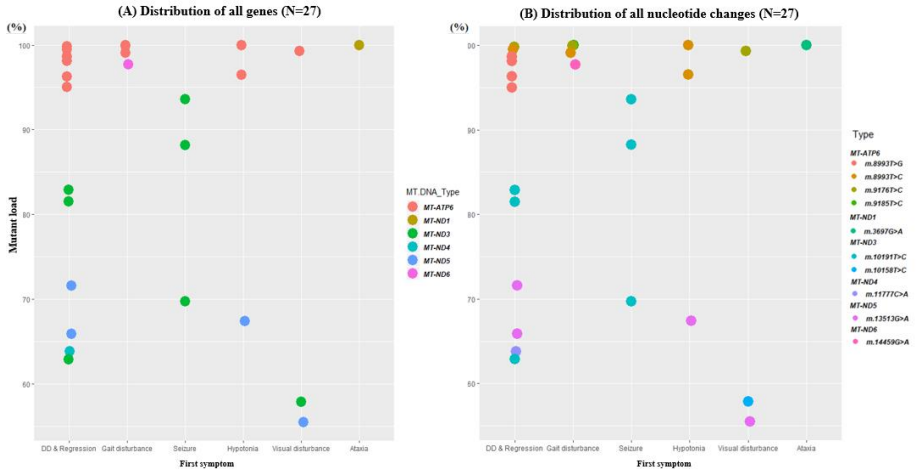

Figure 8. Scatter plot of relationship of mutant load and first symptom.

(A) In the aspect of pathologic variants of mitochondrial genotypes.

(B) In the aspect of pathologic nucleotide changes.

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(3) Relationship of the mtDNA mutant load and lactic acidosis (Figure 9)

A correlation study was performed to investigate the relationship between the mtDNA mutant load and lactic acidosis. The levels of lactic acidosis were measured at the time of diagnosis of Leigh syndrome and quantitatively measured. However, mutant load and lactic acidosis did not have any particular pattern. Also, there was not any particular relationship when looking at genotype and nucleotide change.

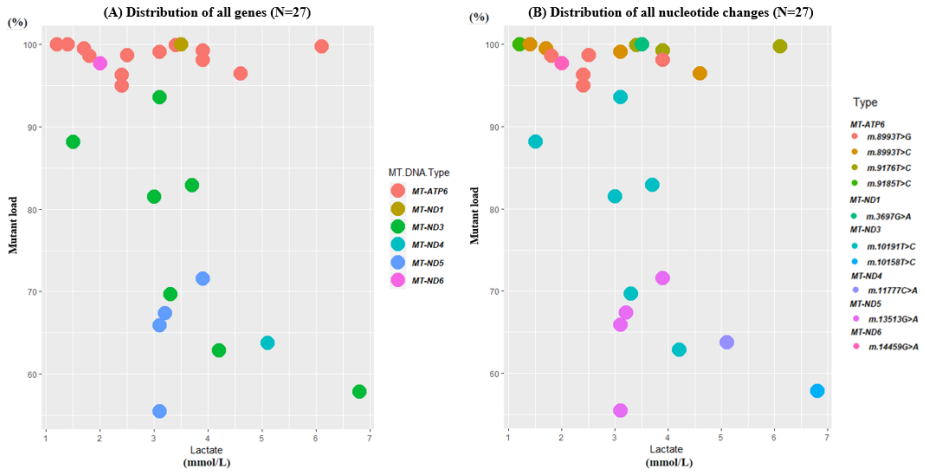

Figure 9. Scatter plot of relationship of mutant load and lactic acidosis.

(A) In the aspect of pathologic variants of mitochondrial genotypes.

(B) In the aspect of pathologic nucleotide changes.

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(4) Relationship of mutant load and organ involvement (Figure 10)

At the time of last follow-up, pattern analysis was performed on the organs of deteriorated function according to mutant load and pathologic variants of mitochondrial genotype / pathologic nucleotide change. As a result, there was no correlation between mutant load and phenotypes. However, pathologic variants of certain mitochondrial genotypes were found to have a relatively high frequency of involvement with certain organs compared to other genotypes. First, CNS involvement was observed in all patients. In particular, *MT-ATP6* and *MT-ND3* patients were involved in most organs. Pathologic variants of *MT-ATP6* were associated with 50% (n = 8) of all patients with deteriorated phenotype of eye (n = 16). The related pathologic nucleotide changes were m.8993T> G (n = 3), m.8993T> C (n = 3) and m.9176T> C (n = 2). However, heart and skeletal involvement were not observed in *MT-ATP6* patients. Of the patients with involvement of the respiratory system, gastrointestinal system and endocrine system (n = 8), 50% of patients were associated with pathologic variants of *MT-ND3*. The pathologic nucleotide change associated with it was m.10191T>C (N = 4). In 75% of *MT-ND5* patients, characteristic cardiologic involvement was observed as a phenotype (n = 3), accounting for 50% of all patients with cardiac involvement (n = 6) and associated pathologic nucleotide change m.13513G>A (n = 3).

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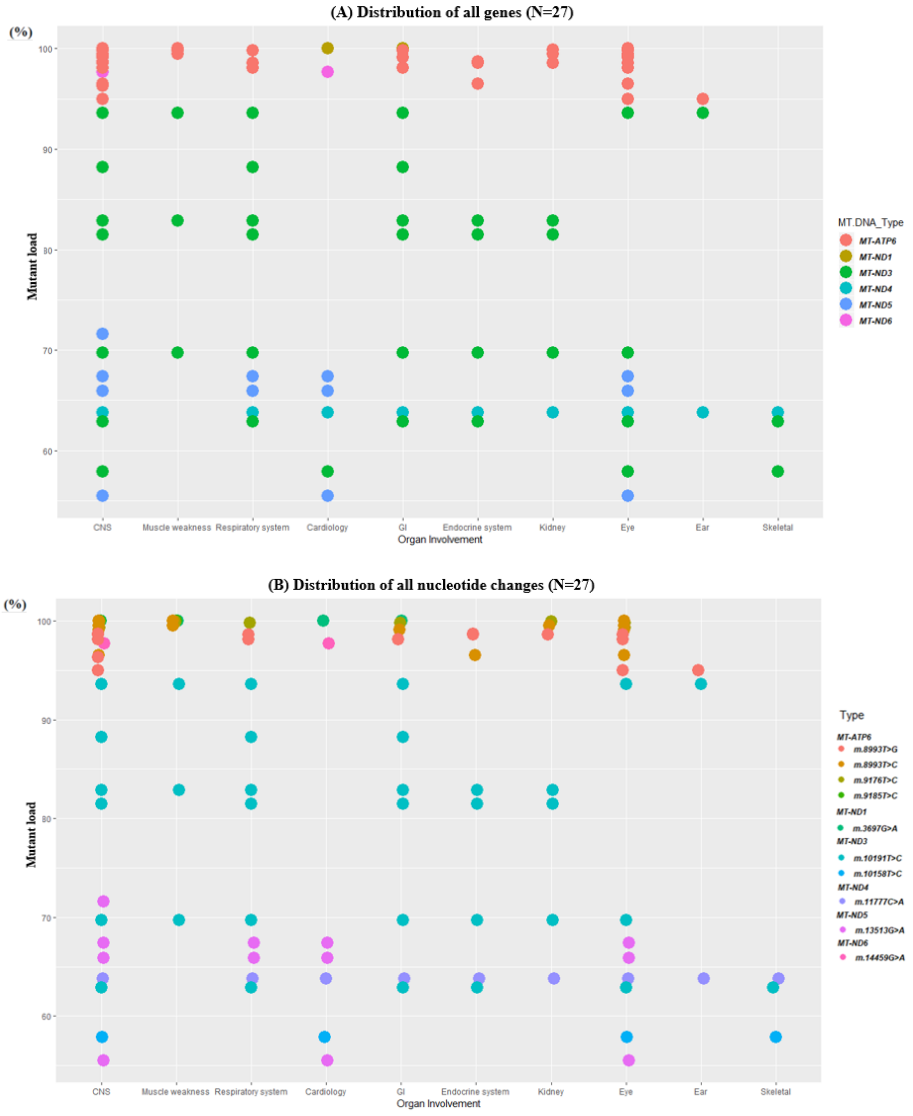

Figure 10. Scatter plot of relationship of mutant load and organ involvement. (A) In the aspect of pathologic variants of mitochondrial genotypes. (B) In the aspect of pathologic nucleotide changes.

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(5) Morbidity of epilepsy (Figure 11)

Additional analyses were performed to further explore the clinical phenotype and organ involvement of Leigh syndrome patients. Epilepsy is one of the main phenotypes that can occur in Leigh syndrome. We analyzed the morbidity of epilepsy as pathologic variants of mitochondrial genotype and pathologic nucleotide change. As a result, morbidity of epilepsy was observed at a very high frequency in *MT-ND3* (n = 6, 85.7%). The major pathologic nucleotide changes related to this were m.10191T> C and pathologic variant of m.10191T> C showed 100% morbidity of epilepsy.

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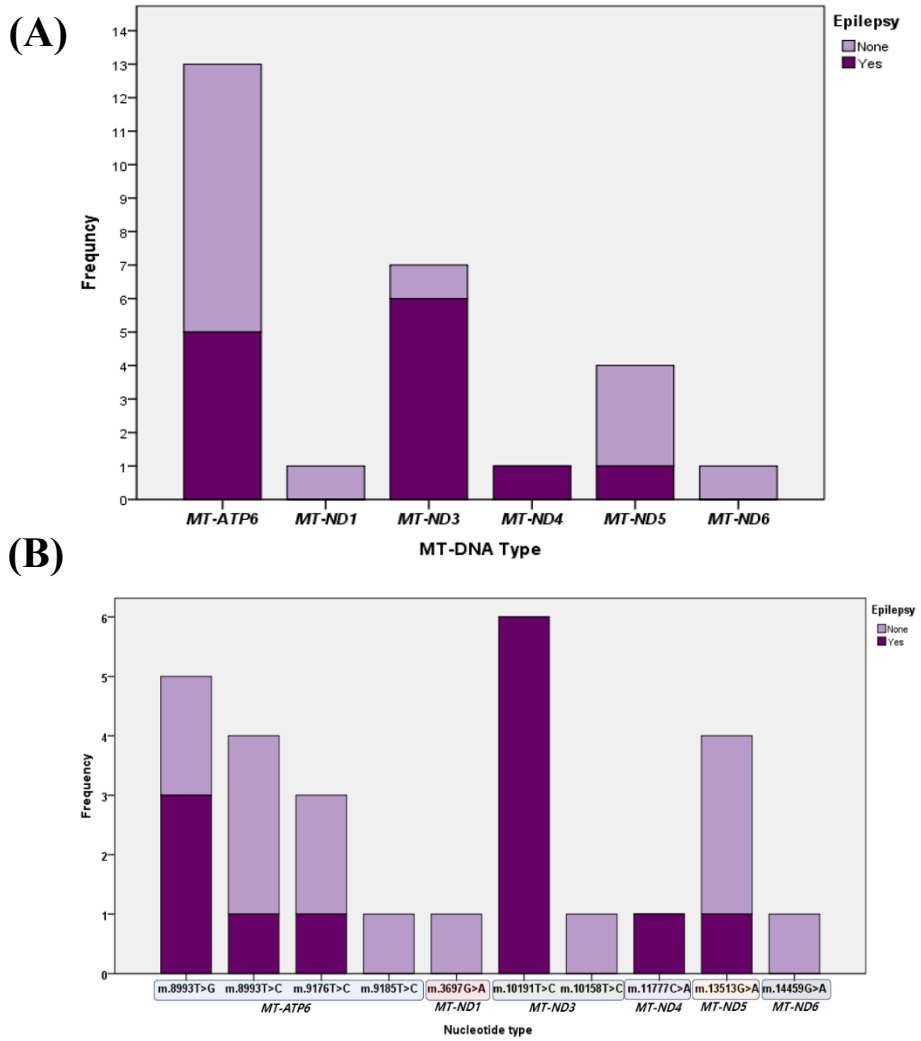

Figure 11. Bar chart of morbidity of epilepsy. *MT-ND3* has a relatively high frequency of epilepsy morbidity. The related pathologic nucleotide change is m.10191T> C, which shows 100% epilepsy morbidity. (A) In the aspect of pathologic variants of mitochondrial genotypes (B) In the aspect of pathologic nucleotide changes.

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(6) Relationship of mutant load and brain imaging (Figure 12, 13)

The following is the relationship between mutant load and MRI involvement. No clear pattern was found for specific genotypes or nucleotide changes and MRI involvement, but extensive involvement of *MT-ATP6* and *MT-ND3* was observed at the genetic level, and brain cortex and white matter involvement were not found in all pathologic variants of the *MT-ND5* gene (m.13513G> A). The frequency of lactate peak was examined by genotype and nucleotide change, and the distribution was relatively evenly distributed without concentrating on a specific genotype or nucleotide change. The related nucleotide change was m.13515G> A. In the MRS, there were no specific deviations from the specific genes or nucleotide changes.

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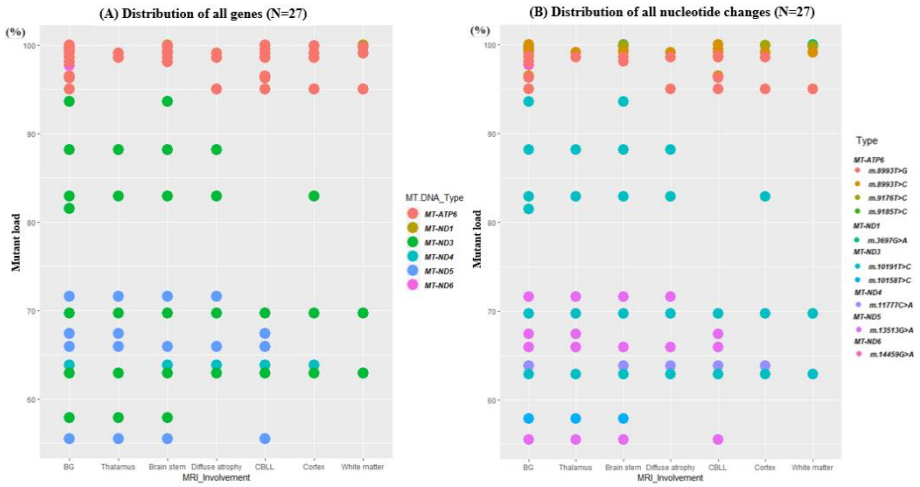

Figure 12. Scatter plot of relationship of mutant load and MRI involvement.

(A) In the aspect of pathologic variants of mitochondrial genotypes.

(B) In the aspect of pathologic nucleotide changes.

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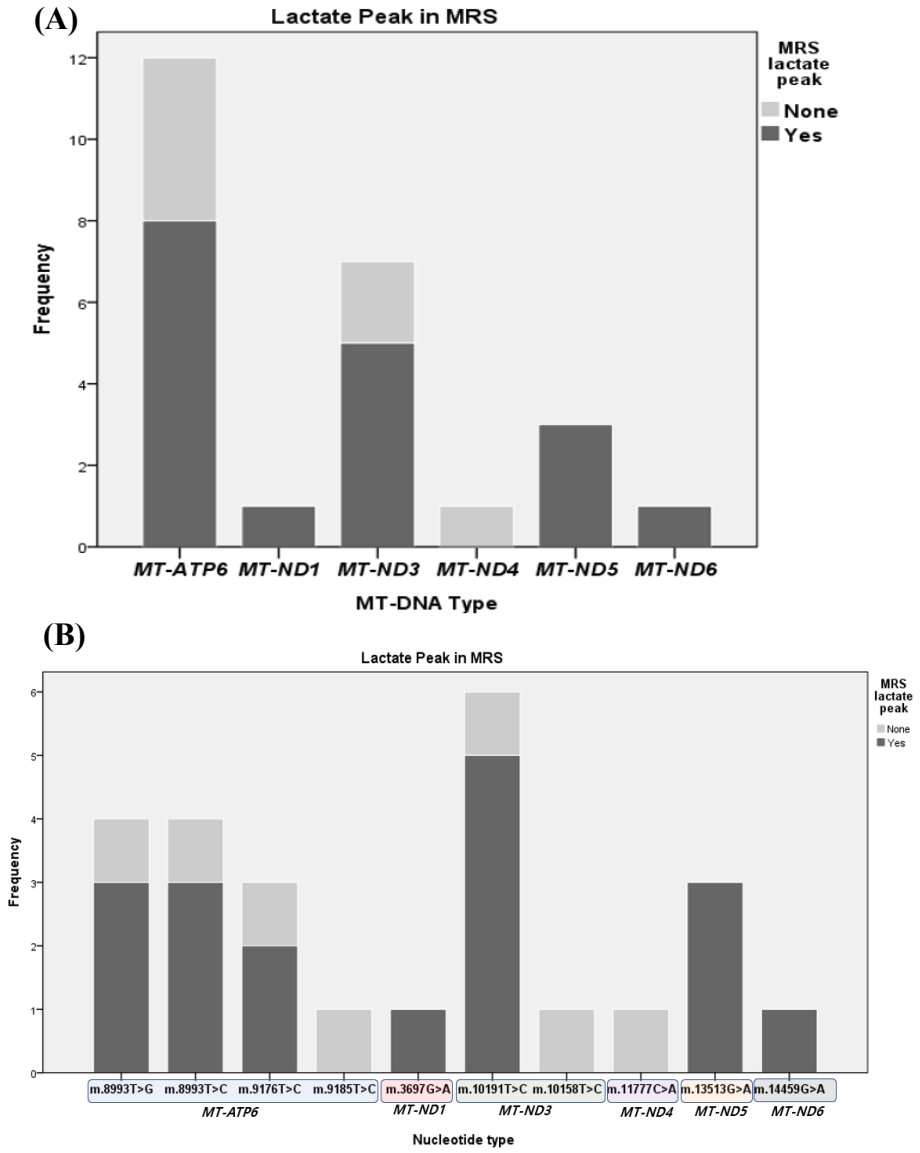

Figure 13. Bar chart of lactate peak in MRS. (A) In the aspect of pathologic variants of mitochondrial genotypes. (B) In the aspect of pathologic nucleotide changes.

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(7) Clinical severity of all genes and nucleotide changes (Figure 14)

We followed up with the patients for a long time (median follow-up period, 5.4 years) and classified their clinical severity into three stages of 'mild', 'moderate' and 'severe'. We compared the prognosis of patients by *MT-DNA* type and nucleotide change. The classification of clinical severity according to the mitochondrial DNA genotype revealed that *MT-ATP6* and *MT-ND5* had a relatively less severe clinical course, whereas *MT-ND3* had a tendency to show a more severe clinical course. Clinical severity was divided into nucleotide changes, and the top five major mutations were examined. As a result, the m.10191T> C mutation was shown as having a relatively more severe clinical status.

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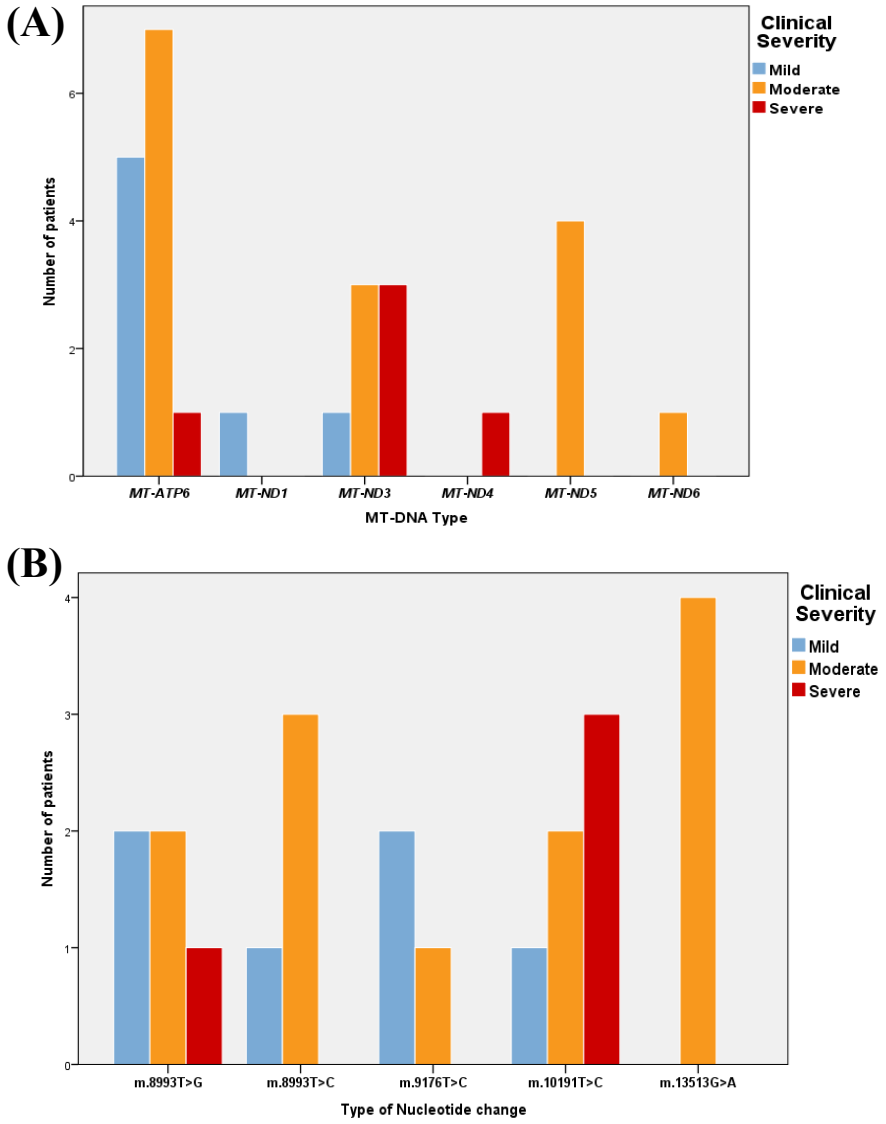

Figure 14. Clinical severity of all patients

(A) Distribution of clinical severities according to mitochondrial DNA genotypes. (B) Distribution of clinical severities according to nucleotide changes of top 5 of all nucleotide mutations.

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(8) Supportive care of the patients (Figure 15, 16)

Patients with Leigh syndrome may require respiratory supports such as a tracheostomy and a home ventilator as the clinical course progresses. In this regard, we investigated whether respiratory support is provided by specific pathologic variants of mitochondrial genotypes or pathologic nucleotide changes. As a result, a relatively high requirement for respiratory supports was observed in *MT-ND3* and *MT-ND5*. The related pathologic nucleotide changes were m.10191T>C and m.13513G>A. These were 66.7% and 50%, respectively, requiring respiratory supports. In addition, oxygen dependency was relatively high in m.10191T> C and m.13513G> A, and a pattern similar to the respiratory support requirement was observed.

Patients with Leigh syndrome may require the use of nasogastric tubes or percutaneous endoscopic gastrostomy (PEG) with / without Nissen fundoplication as the involvement of the gastro-intestinal tract. A relatively high frequency of enteral tube feeding was observed in *MT-ND3* pathologic variant patients. One pathologic nucleotide change related to this finding was m.10191T> C, and among the 9 patients who underwent enteral tube feeding, four patients (44.4%) were pathologic variants of m.10191T> C.

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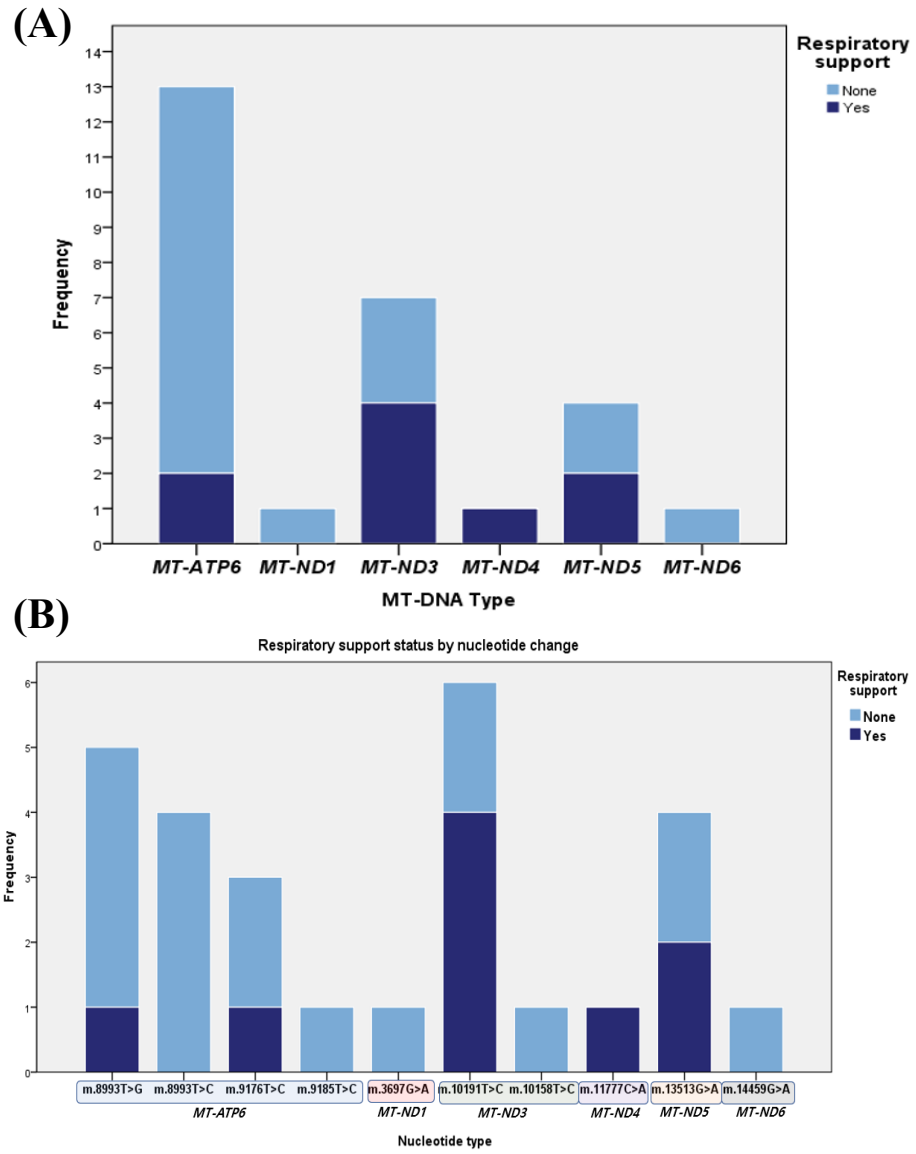

Figure 15. Bar chart of requirement of respiratory support. (A) Requirement of respiratory support in the aspect of pathologic variants of mitochondrial genotypes (B) Requirement of respiratory support in the aspect of pathologic nucleotide changes.

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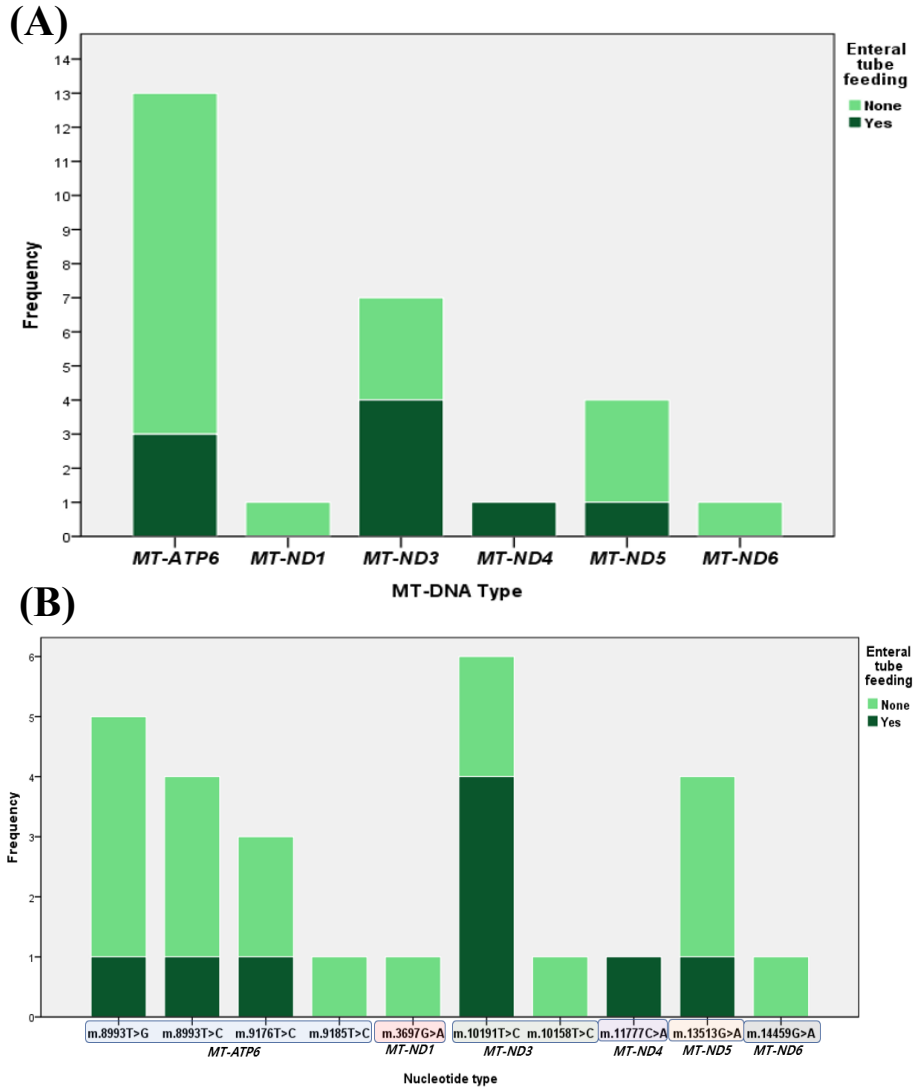

Figure 16. Bar chart of need of enteral tube feeding.

(A) In the aspect of pathologic variants of mitochondrial genotypes. (B) In the aspect of pathologic nucleotide changes.

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IV. DISCUSSION

This study was performed on patients with genetically confirmed mtDNA associated Leigh syndrome. mtDNA associated Leigh syndrome constitutes from 21% to 34% of the total Leigh syndrome, depending on the literature, and the prevalence of mtDNA-associated Leigh syndrome is likely to be 1: 100,000 to 1: 140,000. This is less than nDNA-associated Leigh syndrome, and it is known that nDNA associated Leigh syndrome generally has a more severe clinical course than mtDNA associated Leigh syndrome. [9,17,44] Fourteen genes have been reported to date, which are the key genes involved in causing mtDNA associated Leigh syndrome. A relatively small number of genes have been reported compared to nDNA- associated Leigh syndrome, which accounts for most of Leigh syndrome, and the number of patients is also less than nDNA-assisting Leigh syndrome. Unlike nDNA associated Leigh syndrome, mtDNA associated Leigh syndrome should also be related to heteroplasmy, and the nucleotide mutation site according to the gene is very diverse. Therefore, there are not much research on mtDNA associated Leigh syndrome.[9, 17, 24, 31,44,45]

In our study, there was no specific relationship between heteroplasmy and symptom types, age of first symptom, clinical severity and neuroimaging findings. However, when patients were grouped by mutant gene or mutant nucleotide, heteroplasmy was significantly different in each group. There are 6

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groups of genotypes of Leigh syndrome included in this study, but because there was only one case each of genotypes other than *MT-ATP6*, *MT-ND3* and *MT-ND5*, such as *MT-ND1*, *MT-ND4* and *MT-ND6*, it was difficult to analyze them. Therefore, in this study, it is important to find a specific relationship among *MT-ATP6*, *MT-ND3*, and *MT-ND5* and their associated nucleotide changes and various phenotypes. These three genotypes are known to be the most common genes that cause mitochondrial associated Leigh syndrome, and several recent studies have also reported that *MT-ND3* and *MT-ND5* may exhibit relatively severe phenotypes at low mutant loads, which is consistent with our study.[17,46,47] This means that the threshold value of phenotype expression differs depending on the mutant gene or mutant nucleotide site. Thus, this suggests that we should first consider differences in mutant gene levels when considering heteroplasmy changes in mtDNA, and then study heteroplasmy in the same mutant gene. In mtDNA-associated Leigh syndrome, the distribution of heteroplasmic mutant loads varies with gene or nucleotide change. Therefore, a study with a sufficient number of patients with the same gene or the same nucleotide change would be conclusive and general.[17,24,25,26]

The aim of this study was to investigate the relationship between phenotypes and sequential variables by quantitative analysis of the mutant load at the time of diagnosis and to try to correlate genotypes and phenotypes with pathogenic variants of mitochondrial DNA among Leigh syndrome. Because

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Leigh syndrome is one of rare diseases, it has been difficult to obtain a sufficient number of patients to obtain statistically clear conclusions.[8,9,17] However, considering these limitations, careful subgrouping of various phenotypes such as patient's various symptoms, first symptom of age, first symptom, clinical severity, findings of neuroimaging, muscle biopsy and analysis of association with genotype and mutant load were done. Therefore, we were able to find several meaningful findings that could be helpful in the treatment and prediction of prognosis of the most common genes of mtDNA Leigh syndrome, *MT-ATP6, MT-ND3* and *MT-ND5*.

The main function of the mitochondria is to produce cellular energy in the form of adenosine-5'-triphosphate (ATP). This function is responsible for complex V, the fifth of the five mitochondrial respiratory chains of the mitochondria. It is composed of 15 structural and two assembly subunits, encoded by both the mtDNA (*MT-ATP6* and *MT-ATP8*) and the nuclear genome (15 subunits). Among these subunits, the most common causative gene in the deficiency of Complex V is *MT-ATP6*.[48] The pathologic variant of *MT-ATP6* has been identified as the most common gene causing mitochondrial associated Leigh syndrome and is the most studied genotype. In this study, a pathologic variant of *MT-ATP6* was also found most frequently (N=13). Compared with other genotypes, the *MT-ATP6* gene has a significantly higher amount of mutant load, a wider organ involvement status, and a greater number of first symptoms.

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MT-ATP6 mutations were mostly early onset (< 2 year), but some late onset patients were also observed and wide onset age was observed. According to a recent study by Rebecca D. Ganetzky et al. in which they reviewed all reported mutations of *MT-ATP6* cases (n = 218) to date, they concluded that mutations of the *MT-ATP6* gene have clinical features of a wide range of clinical symptoms, significantly higher mutant load and wide onset manifestations, which are well consistent with our findings.[17,48] It is also noteworthy that pathogenic genotype or nucleotide changes in our study have some specific relationship with various phenotypes, and that patterns are observed in the mutation of *MT-ATP6.* They had relatively often deteriorated function of the eye, but no deterioration was observed in the cardiac and skeletal system in our study. Thus, organ involvement that seems to be characteristic of pathologic variants of *MT-ATP6* may be important in establishing a treatment plan for patients.

MT-ND3 mutation is one of the most common causes of complex I deficiency and Leigh syndrome. Among them, the nucleotide change of m.10191T> C is most reported in the *MT-ND3* mutation. Their clinical spectrum is very diverse and is mostly associated with early onset Leigh syndrome.[48] Nesbitt V et al. reviewed sixteen Leigh syndrome patients with m.10191T> C mutations and concluded that their clinical spectrum was very diverse.[50] In addition, poor clinical correlation was found in their clinical

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severity and heteroplasmic mutant load. This is thought to be due to interaction with other mitochondrial gene mutations or nuclear gene mutations.[49] In this study, the pathologic variant of *MT-ND3* showed a neurologic phenotype despite a relatively lower mutant load than the pathologic variant of *MT-ATP6*. In particular, *MT-ND3* is thought to have a very strong association with epilepsy, and related pathologic nucleotide changes are noted as m.10191T> C. Although there are few reports on the association of epilepsy with Leigh syndrome, Nesbitt V et al. reported seizures in 12 out of 16 patients in m.10191T> C mutations.[50] This is consistent with our findings and may be evidence that an intervention for epilepsy will be needed in patients with the *MT-ND3* mutation, particularly the m.10191T> C mutation. In addition, respiratory supports, oxygen dependency and need for enteral tubes also have a relatively high frequency. This is a finding that can be a good reference for predicting the prognosis of patients with a pathologic variant of *MT-ND3* and preparing them for appropriate treatment.

The *MT-ND5* mutation is also associated with respiratory chain complex I deficiency. In particular, the m.13513G> A mutation is the most common *MT-ND5* mutation. This is known to be caused by mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), but recently it has been linked to Leigh syndrome. This mutation is rarely reported to occur in a relatively lower heteroplasmic mutant load.[13] Brautbar A.

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et al., studied clinical phenotypes of five patients with m.13513G> A. They found a 20-40% lower mutation heteroplasmy, and hypotonia, ocular and cerebellar involvement were frequent.[51] Also, in recent rare case reports, there is evidence that m.13513G> A is symptomatic of arrhythmia, such as, Wolff-Parkinson-White (WPW) syndrome or cardiomyopathy.[14,52.53] In our study, in patients with the pathologic variants of the *MT-ND5* gene (m.13513G> A), a characteristic cardiologic involvement was observed in 75% (N = 3), which is in contrast to the absence of cardiac involvement in *MT-ATP6*. In addition, brain cortex and white matter involvement were not found in these patients. Respiratory supports and oxygen dependency were more frequent in patients with pathologic variants of the *MT-ND5* gene. Thus, the results of this study support the evidence that the *MT-ND5* gene, particularly the m.13513G> A mutation, is characterized by an overall low heteroplasmic mutant load and frequent cardiac involvement. Cardiac involvement in patients with Leigh syndrome is an important risk factor for sudden death, and periodic cardiac access is essential. Cooperative treatment with pediatric cardiology is essential for this.

This study is a well-designed study of Leigh syndrome, which is a rare disease, but it has limitations. Because Leigh syndrome is a rare disease, and there are many areas where statistical analysis is not possible due to the low number of patients. In particular, since patients were divided into subgroups of

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genes, there were statistical limitations due to the smaller number of patients per subgroup. Thus, as implied in this study, when the same genes are clustered, analysis of heteroplasmic mutant load should be followed by studies on mutant load and single mutant genes. However, this study has several advantages over previous studies. This study was limited to mtDNA associated Leigh syndrome which was genetically confirmed by NGS technology, and the relatively large population of mtDNA associated Leigh disease in a single institution were enrolled. Therefore, since it is a single-institution study, the diagnosis, treatment, and overall management of patients are consistent, which makes it more homogeneous than previous studies. In the comparison of previous studies of the mtDNA associated Leigh syndrome, this study has originality in that there are few studies that comprehensively considered about clinical phenotype, gene mutation, pathogenic nucleotide change and heteroplasmic mutant load. In addition, we were able to present some noble findings of mtDNA genotype-phenotype correlations that were not well known in the existing literature. These advantages are likely to be a good reference for the establishment of a tailored treatment plan for mtDNA associated Leigh syndrome. Finally, this is the first attempt to visualize patterns of data that are difficult to process statistically, and this is expected to influence the research methodology of follow-up studies in this area.

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V. CONCLUSION

In our study, we found features of mtDNA Leigh syndrome that were not found in several previous studies. First, the mutant load of the patients at the time of diagnosis is not correlated with the phenotype generally, except for the significant difference of the amount of mutant load among *MT-ATP6*, *MT-ND3* and *MT-ND5*. In other words, the distributions of heteroplasmic mutant loads vary according to the gene, so if we collect enough patients for the same gene or the same nucleotide change, we can conclude a more specific and general conclusion. Second, pathologic variants of mitochondrial genotypes and their associated pathologic nucleotide changes have newly been found to be associated with some phenotypes of mtDNA associated Leigh syndrome.

We investigated various phenotypes of Leigh syndrome by quantitative analysis of heteroplasmic mutant loads according to genotypic subgroups of mtDNA-associated Leigh syndrome which are known to date in order to improve our understanding of Leigh syndrome more deeply. Even though Leigh syndrome is a rare disease, with the increasing number of studies, we may be able to establish a standardized treatment for this disease. This is a further step forward than the previous genotype-phenotype correlation of existing qualitative analysis, and this study would ultimately provide a good basis for interpretation of clinical results, genetic counseling and genetic targeted therapy of Leigh syndrome.

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연세대학교
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ABSTRACT (IN KOREAN)

리이 증후군에서 미토콘드리아 DNA의 돌연변이에 따른 유전자형-표현형 분석

<지도교수 이영목>

연세대학교 대학원 의학과

나지훈

리이 증후군은 소아기의 미토콘드리아 질환의 가장 흔한 질환군이다. 리이 증후군은 미토콘드리아 DNA 연관-리이 증후군과 핵유전자 연관-리이 증후군으로 나뉘는데, 이 둘은 유전형, 임상증상 등에서 큰 차이를 보인다고 알려져 있다. 그 중에서, 미토콘드리아 DNA 연관-리이 증후군은 돌연변이 유전형 그 자체 뿐만 아니라, 이종조직성을 고려해야 하기 때문에 연구에 어려움이 있다. 우리는 미토콘드리아 DNA 연관-리이 증후군에서 돌연변이 부하를 고려하여 유전형-표현형 연관분석을 시행하였다. 우리는 한국의 한 3차 의료기관에서, 임상적으로 리이 증후군으로 진단된 환자 130명에게 차세대 염기서열 분석 기술을 이용하여 전체 미토콘드리아 DNA 염기서열 분석을 수행하였다. 그 결과 27명의 환자들이 유전적으로 미토콘드리아 DNA 연관-리이 증후군으로 진단되었으며, 이들에게 돌연변이 부하를 조사하였다. 또한, 임상적인 특징, 실험실 데이터, 뇌영상들 그리고 장기간 추적검사 데이터를 정리하여 돌연변이 부하와 연관분석을 하였다. 이를 위해서 우리는 데이터 시각화 방법을 이용하여 데이터 패턴을 분석하였다. 전체 미토콘드리아 DNA

연세대학교
YONSEI UNIVERSITY

염기 서열 분석 후, 돌연변이 유전자가 확인된 미토콘드리아 DNA 연관-리이 증후군은 6개의 유전자형, 그리고 10개의 미토콘드리아 DNA 뉴클레오타이드 변이로 분류될 수 있었다. 그 중, 세 가지 주요 유전자의 돌연변이에서 돌연변이 부하 분포의 차이는 통계적으로 유의하게 차이가 있었다. *MT-ATP6* 유전자는 상대적으로 높은 돌연변이 부하를 보인 반면, *MT-ND5* 유전자는 상대적으로 낮은 돌연변이 부하를 보였다. 다른 유전형과 비교하여 *MT-ATP6* 유전자는 돌연변이 부하가 훨씬 높고, 여러가지 장기에 침범하는 양상을 보였다. *MT-ND3*는 뇌전증과 매우 밀접한 관계가 있다고 생각되며, 관련 병리학적 뉴클레오타이드 변화로는 m.10191T>C 가 관찰되었다. 호흡 보조제, 산소 의존성 및 장 튜브와 같은 지지 치료의 필요성은 다른 유전자형보다 *MT-ND3* 유전자 돌연변이 환자에게서 더 높았다. *MT-ND5* 유전자 (m.13513G>A)의 돌연변이를 가진 환자에서 75% (n =3)의 특징적인 심장 관련 침범이 관찰되었는데 이는 *MT-ATP6* 돌연변이에서 심장 관련 침범이 발견되지 않은 것과는 대조적이다. 결론적으로 우리는 이전의 여러 연구에서 발견되지 않은 미토콘드리아 DNA 연관-리이 증후군의 특징을 발견했다. 첫째, 돌연변이 부하의 분포는 유전자에 따라 다르므로 동일한 유전자 또는 동일한 뉴클레오타이드 변화에 대해 충분한 환자를 수집하면 더욱 구체적이고 일반적인 결론을 내릴 수 있다고 생각된다. 둘째, 미토콘드리아 유전형과 뉴클레오타이드의 병리학적 변이가 미토콘드리아 DNA의 일부 표현형과 관련이 있는 것으로 관찰되었다. 통계 분석의 어려움에도 불구하고 이것은 기존의 질적 분석 방법의 유전자형 - 표현형 상관 관계보다 한 걸음 더 나아간 연구이며, 이 연구는 궁극적으로 리 증후군의 임상 결과의 해석, 유전 상담 및 유전자 표적 치료를 위한 기초를 제공할 것으로 기대된다.

연세대학교
YONSEI UNIVERSITY

핵심되는 말 : 리이 증후군, 미토콘드리아 DNA, 유전형, 표현형, 이종조직성, 미토콘드리아 질환, 돌연변이 부하