Mitochondrial DNA mutant load of A3243G mutation and its clinical correlation

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Directed by Professor Young-Mock Lee

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Ha Neul Lee

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This certifies that
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<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Mitochondrial DNA mutant load of A3243G mutation and its clinical correlation

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Background: Mitochondrial disorders are a group of genetically and clinically heterogenous disorders resulting from mutations in the nuclear or mitochondrial DNA (mtDNA), causing dysfunction, and resultant defective energy production. Among them, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) is one of the most commonly inherited types of encephalopathy of maternal origin; that occurs through mutation in mtDNA. MELAS is characterized by ongoing and degenerative disease course because of recurrent stroke-like episodes and associated neurologic sequelae, involvement of other non-neurologic organs including the heart, kidney, gastrointestinal system, endocrine system, and even psychological issues. Around ~80% of MELAS patients harbor one specific mutation in mtDNA, A to G transition of tRNA gene for leucine (UUR) at nucleotide position 3243. Disease-causing mtDNA mutations are heteroplasmic, which implies that normal and mutant mitochondria are mixed within the same cells. The level of heteroplasmy, or in other words, mutant load, can vary in affected individuals, which contributes to extreme clinical variability. Several previous studies have investigated the natural history and disease progression of the MELAS syndrome, yet correlation

between mtDNA mutant load and disease progression in MELAS is not completely understood. Therefore, I aimed to investigate the levels of blood mtDNA A3243G mutant load at the time of genetic diagnosis via next generation sequencing (NGS) technology of patients confirmed to have pathogenic A3243G mutation, and then to investigate the correlation of blood mtDNA mutant load and functional scale changes over time to reflect disease progression in MELAS. I also investigated the correlation, especially with respect to the neurological and muscular aspects in consideration of recurrent stroke-like episodes.

Methods: I performed whole mitochondrial DNA sequencing by NGS on samples from 57 patients with clinical suspicions of MELAS syndrome. Among them, 32 patients were confirmed to have mtDNA A3243G mutation. Finally, 25 patients who met the clinical critieria for MELAS, had mtDNA A3243G mutation and blood samples available for mtDNA mutant load analysis at the same time, were recruited for final inclusion. I collected the demographics and clinical data by retrospective electronic medical chart review. I applied functional scales that were previously published and validated in several other studies, including modified Rankin Scale (mRS), The Newcastle Paediatric Mitochondrial Disease Ratings Scale (NPMDS), and The Newcastle Mitochondrial Disease Adult Scale (NMDAS). As these scales contain large number of questions and with wide score range, especially NPMDS and NMDAS, I modified and simplified the questions from these questionnaires and designed functional scales for the current study. Function_total, Neuromuscular_total, and Non-neuromuscular_total are 3 simplified and modified versions. I applied these 6 functional scales for 25 MELAS patients at 4 different time points during the disease progression – symptom onset, first year, second stroke-like episode and the last visit. Then I analyzed the correlation between the mtDNA mutant load at genetic diagnosis and obtained changes in functional scale over time. P-value <.05 was considered statistically significant.

Results: Quantitative analysis of mtDNA mutant load at genetic diagnosis via NGS

method revealed the mean mutant load of 60.2±18.8% (22.5-100). I investigated the association between the mtDNA mutant load at genetic diagnosis and clinical variables, and functional scale changes at 4 different time points to reflect the degenerative disease course of MELAS. This study revealed that the mutant load at genetic diagnosis inversely correlated with age of symptom onset, age at seizure onset, and age at first clinical and MRI-confirmed stroke-like episode (all *P*<.0001, respectively). The mtDNA mutant load also negatively correlated with worse abnormality in MRS study and maximal serum lactic acidosis level (P=0.0032 and P=0.0007, respectively). When I analyzed the correlation between mtDNA mutant load and functional scales, the mtDNA mutant load did not significantly correlate with any of the 6 functional scales. When I looked further into each time point, Function_total correlated positively at symptom onset (r=0.5476, P=0.0046) but this trend did not persist through disease progression. Neuromuscular_total did not correlate significantly at symptom onset but it correlated positively with significance at the last visit (r=0.4418, P=0.027). Therefore, I further investigated the changes in functional scales in between each time point and their correlation with the mutant load, and I found that the changes in Neuromuscular_total (Δ) during disease progression (symptom onset – year 1- 2nd stroke-like episode – last visit or symptom onset – year 1 – last visit or symptom onset – 2nd stroke-like episode – last visit) all correlated positively with significance (r=0.5075, P=0.0096; r=0.532, P=0.0062; and r=0.4698, P=0.0178, respectively). When I investigated further to examine if this trend continued with increase in disease duration by 1 month, the trend was consistent with statistical significance (r=0.4284, P=0.0326, r=0.428, P=0.0328, r=0.4012, P=0.0468, respectively).

Conclusion: In conclusion, the blood mtDNA mutant load obtained at the time of genetic diagnosis (earlier in the disease course and close to first clinical stroke-like episode) in clinically symptomatic patients who were confirmed to have a mtDNA A3243G pathogenic mutation with a genetic diagnosis, the higher mutant load the earlier symptom onset, seizure onset, and stroke-like episode age, reflected worse

clinical severity from the beginning. In addition, even though previously validated functional scales of mRS, NPMDS, and NMDAS reflect changes with time in these population with significance, yet they do not correlate with blood mutant load at genetic diagnosis. However, modified and simplified versions of Neuromuscular_total reflected disease progression with significant correlation with blood mutant load, which may enable better clinical decision making and provisions for expected counseling in these patients. The current study data was limited by the lack of validity of the modified and simplified versions of our functional scales which need further validation with a larger number of mitochondrial disease patients, and not only those with MELAS.

Keywords: MELAS; mitochondrial disease; A3243G mutation; mutant load; heteroplasmy; neuromuscular function

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I. Introduction

Mitochondrial disorders originate from mutations in the nuclear or mitochondrial deoxyribonucleic acid (DNA), and consequently the mitochondrial respiratory chain dysfunction leads to defective energy production. Complicated inheritance and penetrance pattern, and different energy demand of each organ contributes to extreme heterogeneity in these groups[1].

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) was first described by Pavlakis et al. in 1984[2] and is one of the most common maternally inherited types of encephalomyopathy. Clinically, MELAS patients suffer encephalopathy that is frequently accompanied by epileptic seizures, migrane-like headache and stroke-like episodes occurring before the age of 40 years and sometimes in childhood, and serum lactic acidosis. Developmental delay, mental retardation, dementia, seizures, status epilepticus, tremor, ataxia, dystonia,

neuropathy, muscle weakness and visual disturbances may also develop, with respect to the neurological parameters. Not only that, various other organ systems may also be involved, leading to pigmentary retinopathy, optic atrophy, sensorineural hearing loss, gastrointestinal involvement, hypertrophic cardiomyopathy, arrhythmia, respiratory issues, renal involvement, short stature, diabetes mellitus and even psychological issues[3-7]. Altogether, variable degree of involvement of each organ results in extreme clinical heterogeneity in MELAS patients. In addition, recurrent stroke-like episodes further contribute to clinical deterioration[3]. As the disease progresses, literally all aspects of daily life are affected such as mobility, education, social/vocational life and self-care.

About 80% of MELAS patients harbor one specific mutation in the mitochondrial DNA, A to G transition of tRNA gene for leucine (UUR) at nucleotide position 3243[3,7-10]. Even with this particular mutation, clinically diverse spectrum develops, which makes genotype-phenotype correlation and genetic counseling even more difficult[7-13]. As MELAS patients suffer chronic degeneration over time, attempts to fully understand the natural history of this disease have been made in previous studies; in relation to A3243G mutation itself and the level of heteroplasmy, or in other words, mutant load[14-21]. Disease-causing mtDNA mutations are heteroplasmic, which implies that normal and mutant mtDNA are mixed within the same cells in a random way. This variability of mutant load exists not only at a cellular level but also at organ and individual levels, which further complicates and confers extreme heterogeneity even in siblings and relatives carrying the same mutation from the same family tree[14,16,17].

There have been several methods to detect the A3243G mutation itself and analyze the mutant load[22-33], and among them the next generation sequencing (NGS) method has shown promise in diagnosing patients with mitochondrial disease in the context of extreme clinical and genetic heterogeneity when not meeting specific clinical criteria for certain mitochondrial syndromes[23-30]. The NGS method can also be used to evaluate the mutant load simultaneously, which decreases the burden of labor and expenses with ease. Considering this, similar studies had tried to investigate the

natural history of MELAS considering the A3243G mutation itself and the mutant load, but with no clear cut conclusion so far[16-21].

Therefore, in this study, I aimed to quantify the mtDNA A3243G mutant load via NGS method in clinically symptomatic patients with MELAS who were confirmed to have pathogenic A3243G mutation to narrow down and focus on the homogenous group of patients. I selected to quantify the A3243G mutant load at a certain time point of genetic diagnosis, which approximates earlier in the disease course in most patients. In addition, I hypothesized that there would be a correlation between the blood mtDNA A3243G mutant load at the time of genetic diagnosis and clinical disease severity and progression, especially in terms of neuromuscular function. To objectively evaluate the functional status at each time point in the disease progression, I evaluated general functional status, neuromuscular manifestation and other organ involvement using previously known and validated functional scale scores, and simplified and modified versions designed for the current study. In conclusion, I aimed to investigate the relationship between the blood mutant load at genetic diagnosis and functional changes with disease progression in MELAS patients in a single tertiary center.

II. MATERIALS AND METHODS

1. Patient selection

Figure 1 describes the patient selection criteria. I first selected patients meeting the diagnostic criteria by Bernier et al. and with clinical symptoms compatible with MELAS who were diagnosed and regularly followed at Gangnam Severance Hospital, Department of Pediatrics from 2010 to 2019. Of 57 patients selected, 32 were confirmed to have pathogenic mtDNA A3243G mutation by blood genetic testing. Among these 32 patients, the blood mtDNA A3243G mutant load (heteroplasmy) analysis was available in 25 patients at the same time with the genetic diagnosis. Therefore, 25 subjects positive for A3243G mutation and available mutant load

results were finally recruited for the study.

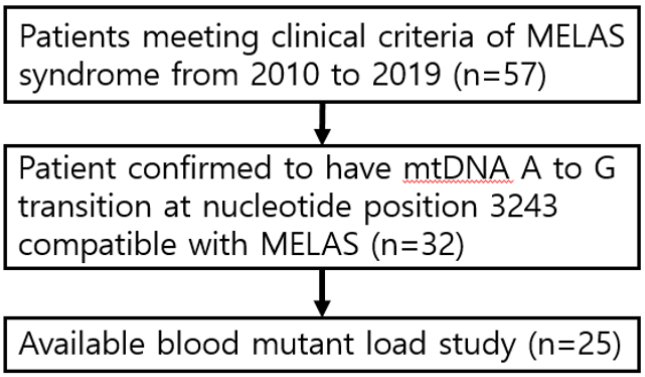


Figure 1. Patient selection criteria

2. Genetic confirmation of the A3243G mutation and mutant load analysis by next generation sequencing (NGS) method

A. Genetic confirmation of the mtDNA A3243G mutation

(1) Sample preparation

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

(2) Library preparation and sequencing

The PCR product was fragmented into 150 to 200 basepair (bp) segments using a NEBNext dsDNA Fragmentase® (New England Biolabs, UK), according to the

manufacturer's protocol. The enzyme-fragmented PCR products were 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, USA). Libraries were sequenced by synthesis on MiSeq for paired 150 bp read lengths using Illumina miSeq V3 Kits (Illumina, USA).

(3) Analysis of sequence variants

The sequenced reads were mapped to the human mitochondria reference sequence (NC_012920) with Burrows-Wheeler Aligner software and the variants were identified with the Genome Analysis toolkit. Sequence variants were filtered according to quality parameters.

B. Quantitative analysis of blood A3243G mutant load (heteroplasmy)

Quantitative analysis for blood mutant load was performed by counting the number of reads for each sequenced template by NGS. The mtDNA mutant load percentage measurement was obtained using mtDNA navigator software, which converted automatically from the read data (Figures 2-1 and 2-2).

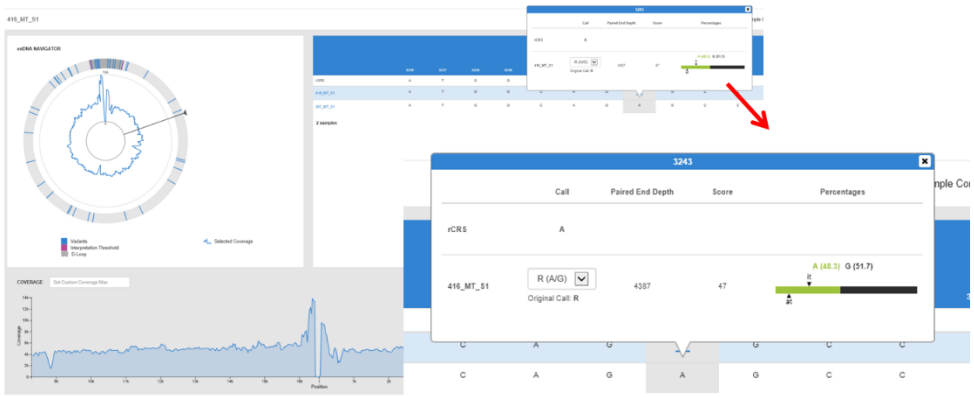


Figure 2-1. The mtDNA navigator software and automatic calculation of mtDNA mutant load percentage

Figure 2-2. The mtDNA mutant load percentage in a MELAS patient and a normal subject

3. Data collection

A. Clinical data collection

A retrospective chart review of electronic medical records was done in 25 subjects. The demographics and clinical information were collected. The age of onset, initial presenting symptom, age at 1st clinical and MRI-confirmed stroke-like episode, age at genetic diagnosis of MELAS, and follow up duration data were recruited.

B. Diagnostic evaluations of mitochondrial disease

Laboratory tests including maximal serum lactate values and accordant pyruvate values were reviewed. Muscle biopsy data and biochemical enzyme assay results were also reviewed.

Imaging studies including brain magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) were reviewed. Regional signal changes and atrophy data, and infarct data were collected for MRI, whereas lactate peak, decreased NAA and increased choline peak were reviewed for MRS study.

C. Application of functional scales to evaluate disease severity

To evaluate clinical disease severity, we tested 3 previously reported functional scales that were validated in other studies including Modified Rankin Scale (mRS)[34-38], The Newcastle Paediatric Mitochondrial Disease Ratings Scale (NPMDS)[39], and The Newcastle Mitochondrial Disease Adult Scale (NMDAS)[40]. The details on each scale are described in a latter section. In addition, I also tested 3 scales that were designed particularly for this study, Function_total, Neurouscular_total and Non-neuromuscular_total. These 3 scales and detailed scoring have also been described in the latter part of this thesis. To investigate the changes with time progression, I applied these six functional scales to 4 different time points in MELAS natural history. Symptom onset, year 1 from the symptom onset, 2nd stroke-like episode (N=21), and the last visit (3 other time points, N=25).

D. Application of previously reported and validated functional scales

(1) Modified Rankin Scale (mRS)

The modified Rankin Scale (mRS) is the most prevalent functional outcome measure in contemporary stroke research[34]. mRS is a clinician-reported measure of global disability that has been widely applied for evaluating recovery from stroke[35-37]. Clinical trial use of mRS is expanded to a primary end point in randomized clinical trials of stroke treatments. mRS is also widely used in studies regarding acute ischemic stroke in children and young adults[38]. The mRS scores are as follows: scores from 0 (no symptoms at all) to worst function of maximum 6 (dead); as the score increases, disability regarding mobility and self-care worsens.

(2) The Newcastle Paediatric Mitochondrial Disease Rantings Scale (NPMDS)

The Newcastle Paediatric Mitochondrial Disease Rantings Scale (NPMDS) was designed and published by Phoenix et al. in 2006[39], and was developed to objectively monitor the mitochondrial disease progression in children. This scale had been used in several previous studies to reflect the disease severity and progression, and is multi-dimensional and reproducible. This scale contains 4 sections; 1, function (7

questions); 2, system specific involvement (10 questions); 3, current clinical assessment (9 questions); 4, quality of life; and each question score from 0 (normal) to 3 (severe). This scale also has 3 separate age ranges: 1, 0-2 years; 2, 2-11 years; and 3, 12-18 years. Among these, I selected 2-11 years and 12-18 years versions according to our patients' age range, and score from section 1-3, apart from section 4, quality of life. Total score of section 1-3 ranged from 0 to 78. Total score of neurology related questions ranged from 0 to 30.

(3) The Newcastle Mitochondrial Disease Adult Scale (NMDAS)

The Newcastle Mitochondrial Disease Adult Scale (NMDAS) was designed and published by Schaefer et al in 2006[40] by the same Newcastle group for objective clinical rating of mitochondrial disease in adults and to monitor disease progression. This scale also contains 4 sections; 1, function (10 questions); 2, system specific involvement (9 questions); 3, current clinical assessment (10 questions); 4, quality of life; and each question score from 0 (normal) to 5 (unable). Each question and section contains slightly different contents compared to NPMDS but they are still almost similar. Self-care and handling part are more detailed. This scale does not have separate age range, and thus is available for all age ranges after 18 years. Total scores of sections 1-3 ranged from 0 to 145. Total scores of neurology related questions ranged from 0 to 75.

E. Application of modified version of functional scales designed in the current study

As the above mentioned NPMDS and NMDAS scales contain large number of questions covering the whole spectrum of daily living and function, I wanted to design simpler scale to evaluate the disease severity and monitor the disease progression.

(1) Function_total

I designed the scale "Function_total", which includes motor, verbal and social

function, and scored sum as follows:

Motor: 0 (normal), 1 (mild; ambulatory and/or independent for daily activities), 2 (moderate; wheelchair bound and/or partially dependent for daily activities), 3 (severe; bedridden and totally dependent for daily activities).

Verbal: 0 (normal), 1 (mild; able to communicate even in a few words), 2 (severe; unable to communicate).

Social: 0 (normal or appropriate for age), 1 (mild; difficulties at school or work yet maintains ability), 2 (severe; not able to perform schoolwork or job).

Total score ranged from 0 to 7.

(2) Neuromuscular_total

I designed scale "Neuromuscular_total" including total 14 aspects of neurologic and muscular involvement in MELAS patients: developmental delay, mental retardation, dementia, epilepsy, recent seizure, status epilepticus event, intractable epilepsy using ≥3 antiepileptic drugs, ambulation ability, skeletal muscle weakness involving both upper and lower extremities, stroke-like episodes, headache/migrane, ataxia, tremor, and visual disturbance associated with stroke-like episodes. All categories scored either 0 (no) or 1 (yes), apart from the ambulation ability: 0 (normal), 1 (affected but able), and 2 (severely affected and unable). Total score ranged from 0 to 15.

(3) Non-neuromuscular_total

I designed scale "Non-neuromuscular_total" to evaluate the involvement of total 11 organs apart from neurological and muscular involvement. Categories are as follows: psychologic issues, respiratory involvement (normal, oxygen dependent, noninvasive ventilation, tracheostomy and using ventilator support), cardiac function, presence of arrhythmia, glucose intolerance/diabetes mellitus, other endocrine involvement including short stature or hypothyroidism, gastrointestinal involvement including severe constipation/diarrhea, feeding (normal, G-tube placement, and

gastrostomy placement), renal involvement, eye involvement (pigmentary retinopathy and optic atrophy), and sensorineural hearing loss. Total score ranged from 0 to 15.

4. Statistical analysis

Statistical analysis was performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Data are presented as mean, standard deviation, median, and ranges. The Pearson correlation was used to evaluate correlation between blood mutant load and clinical variables and functional scale scores. Linear mixed model was used to evaluate differences in functional scale scores at 4 different time points (symptom onset, year 1, 2nd stroke-like episode, last visit). The linear regression method was used to evaluate the slope of changes in functional scale scores at 4 different time points, and then the Pearson correlation was once again used to evaluate correlation between the blood mutant load and changes in slope during disease progression. *P*-value <.05 was considered statistically significant.

5. Study design summary

Figure 3 summarizes study design as a whole. Briefly, 25 patients were selected for the final study and all were confirmed to have pathogenic mtDNA A3243G mutation and available blood mutant load study simultaneously. Altogether 6 functional scales were applied at 4 different time points in the MELAS disease progression; symptom onset, year 1, 2nd stroke-like episode, and the last visit. Three previously known scales, mRS, NPMDS, and NMDAS and simplified and modified versions designed for my study, Function_total, Neuromuscular_total and Non-neuromuscular_total, were applied. Investigation of correlation between blood mutant load at genetic diagnosis and functional scale scores according to disease progression was performed.

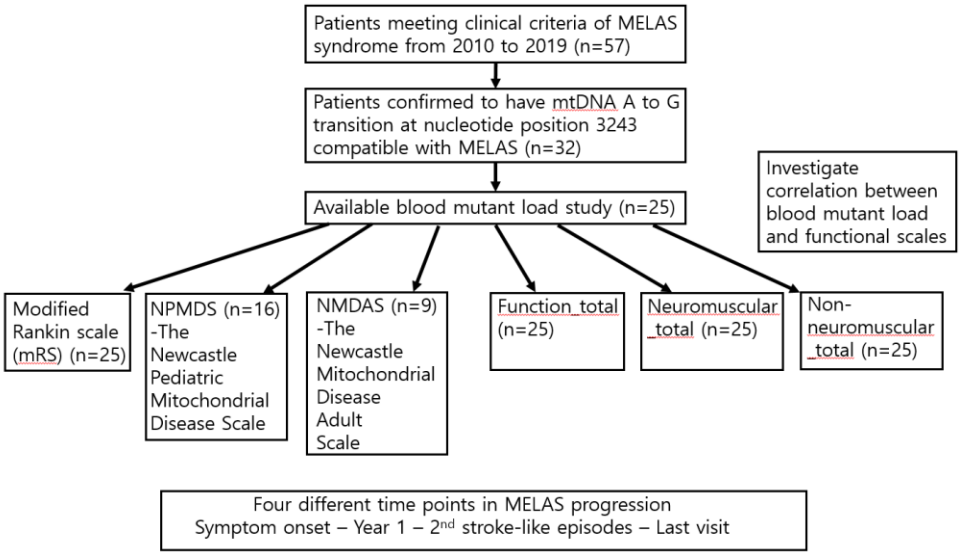


Figure 3. Study design summary

III. RESULTS

1. Patient demographics and general characteristics

Table 1 summarizes the patient demographics and general characteristics. Of 25 MELAS patients, 14 were males and 11 were females, and the age of onset was 13.2±8.4 years (range: 3.5-37.3 years). Initial presenting symptoms were as described, and stroke-like episode was the most common presenting symptom; in 19 of 25 (76.0%) patients. Headache and seizure followed. Not all patients were confirmed with brain MRI evidence of infarct when their first clinical stroke-like episodes happened, showing younger mean age of 1st clinical stroke-like episode at 13.7±8.5 years (range: 3.5-37.3 years) compared to the age of 1st MRI-confirmed stroke-like episode (for some patients this was their 2nd stroke-like episode event) at 14.5±8.4 years (range: 4.1-37.3 years). Age at genetic confirmation of A3243G mutation was 15.2±8.8 years (range: 3.0-37.3 years), and the blood leukocyte at that time was used for mtDNA A3243G mutant load analysis.

Of 25, 20 patients were alive at their last visit at an age of 21.5±8.6 years (range: 5.6-41.3 years) whereas 5 patients deceased at age 20.4±8.9 years (range: 13.8-35.4 years). Follow up duration for all subjects were 8.1±4.4 years (range: 1.7-17.2 years).

Table 1. Patient demographics and general characteristics

Characteristics	**N=25**
Gender (male: female)	14:11
Age of onset (years)	13.2±8.4 (3.5-37.3)
Initial presenting symptom	
Stroke-like episode	19/25 (76.0%)
Headache	11/25 (44.0%)
Seizure	10/25 (40.0%)
Visual disturbance	7/25 (28.0%)
Loss of consciousness	5/25 (20.0%)
Muscle weakness	3/25 (12.0%)
Delayed development	3/25 (12.0%)
Age at diagnosis of MELAS (years)	15.2±8.8 (3.0-37.3)
Age at 1st clinical stroke-like episode (years)	13.7±8.5 (3.5-37.3)
Age at 1st MRI-confirmed stroke-like episode (years)	14.5±8.4 (4.1-37.3)
Long-term follow up result	
Follow up duration (years)	8.1±4.4 (1.7-17.2)
Alive: Deceased	20:5
Age at the last visit if alive (years)	21.5±8.6 (5.6-41.3)
Age at death (years)	20.4±8.9 (13.8-35.4)

2. Diagnostic evaluations of mitochondrial disease

Diagnostic evaluations of mitochondrial disease including the laboratory tests, muscle biopsy and biochemical enzyme assays, and imaging studies including brain MRI and MRS are described in detail in Tables 2 and 3.

Table 2 summarizes the laboratory test results and results of the muscle biopsy, and

biochemical enzyme assays. All 25 patients (100%) were confirmed with pathogenic mtDNA A3243G mutation by blood at age 15.2±8.8 (range: 3.0-37.3) years, and the blood leukocyte at that time was used for mtDNA A3243G mutant load analysis. Blood mtDNA A3243G mutant load was 60.2±18.8 (range: 22.5-100%). All patients had serum lactic acidosis and 64% of the patients (16 of 25) had more than 4 fold elevation than the normal values.

The muscle biopsy data are as shown in Table 2. Not all patients obtained muscle biopsy as all patients were easily diagnosed with blood genetic mutation testing for mtDNA A3243G, they met clinical criteria for MELAS, sparing the need for muscle biopsy for diagnostic reason. Among 13 patients who obtained muscle biopsy, 5 (38.5%) had abnormal light microscopy results and all had ragged-red fiber. Electron microscopy data showed either pleoconia or megaconia. The biochemical enzyme assay data was available for 9 patients (69.2%) and 8 (88.9%) had complex 1 deficiency whereas 1 (11.1%) had complex IV deficiency.

Table 2. Laboratory testing, muscle biopsy and biochemical enzyme essay for diagnosis of mitochondrial disease

Variables	**N=25**
mtDNA A3243G mutation (+)	25/25 (100%)
Blood mtDNA A3243G mutant load	60.2±18.8% (22.5-100)
Laboratory tests	
Serum lactic acidosis	
Normal	0/25 (0%)
Mild elevation (≥2 fold)	5/25 (20.0%)
Moderate elevation (≥3 fold)	4/25 (16.0%)
Severe elevation (≥4 fold)	16/25 (64.0%)
Maximal absolute value of serum lactate (mmol/liter)	8.3±4.3 (2.3-18.5)
Absolute value of serum pyruvate (mmol/liter)	0.20±0.08 (0.06-0.32)
Absolute value of serum lactate/pyruvate	61.7±33.5 (13.2-161.3)
Serum lactate/pyruvate >20	23/25 (92.0%)
Muscle biopsy	13/25 (52.0%)

Age at muscle biopsy (years)	13.6±9.3 (6.4-37.6)
Light microscopy (+)	5/13 (38.5%)
Specific findings	5/5 (100%)
Non-specific findings	0/5 (0%)
Electron microscopy (+)	5/13 (38.5%)
Pleoconia	3/5 (60.0%)
Megaconia	5/5 (100%)
Biochemical enzyme assay	9/25 (36.0%)
Complex I deficiency	8/9 (88.9%)
Complex IV deficiency	1/9 (11.1%)

Table 3 summarizes the study results of imaging. All patients obtained imaging studies, either brain MRI or MRS. Detailed data are shown; of 25 patients who obtained brain MRI, 23 (92.0%) showed MRI evidence of infarct, a likely sequalae from stroke-like episodes.

Table 3. Imaging studies for diagnosis of mitochondrial disease

Diagnostic evaluations	**N=25**
MRI	N=25 (100%)
Evidence of infarct	23/25 (92.0%)
Cortex signal abnormality	23/25 (92.0%)
White matter signal abnormality	20/25 (80.0%)
Ventriculomegaly	20/25 (80.0%)
Basal ganglia signal abnormality	17/25 (78.0%)
Thalamus signal abnormality	9/25 (36.0%)
Caudate nucleus signal abnormality	5/25 (20.0%)
Cerebral atrophy	4/25 (16.0%)
Cerebellar atrophy	19 (79.2%)
MR spectroscopy	N=24 (96.0%)
Presence of lactate peak	18/24 (75.0%)
Decreased NAA	18/25 (75.0%)
Increased choline peak	19/25 (79.2%)

3. mtDNA A3243G mutant load in blood at the time of genetic diagnosis

As mentioned earlier, all 25 subjects were confirmed to have pathogenic mtDNA A3243G mutation by blood testing, and A3243G mutant load obtained at the same time revealed 60.2±18.8 (range: 22.5-100%), at age 15.2±8.8 (range: 3.0-37.3) years. Figure 4 shows % blood mutant mtDNA load in patients according to age at diagnosis and shows inverse correlation between age and mutant load by the Pearson correlation (r=-0.737, *P*=.000).

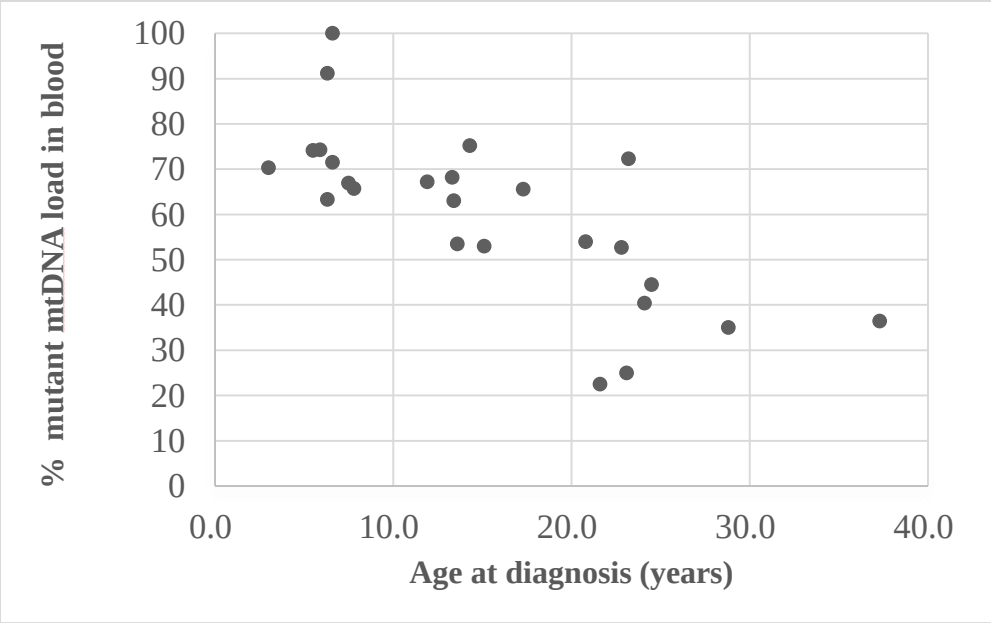


Figure 4. mtDNA A3243G mutant load in blood

4. Functional outcome scale scores according to disease progression

Altogether 6 different functional scales were applied at 4 different time points, symptom onset, year 1, 2nd stroke-like episode (n=21), and the last visit, to investigate the effect of disease progression. Three previously known and validated scales, mRS (n=25), NPMDS (n=16), and NMDAS (n=9) were applied in addition to simplified and modified versions, Function_total (n=25), Neuromuscular_total (n=25), and

Non-neuromuscular_total (n=25).

Table 4 and Figure 5 showss mRS score trend with respect to changes with time in in MELAS. The mRS score ranged from 0 (normal) to 6 (dead), and higher score indicated worsening function. The mRS score increased over time with statistical significance (P<.0001), reflecting worsening function with disease progression.

Table 4. modified Rankin scale (mRS) scores with disease progression

	Modified Rankin scale score (n=25)
Symptom onset (n=25)	1.0±1.0 (0-4)
Year 1 (n=25)	1.0±1.2 (0-4)
2nd stroke-like episode (n=21)	2.0±0.9 (1-5)
Last visit (n=25)	3.0±1.6 (1-5)
P-value	<.0001*

**P*-value was calculated using linear mixed model.

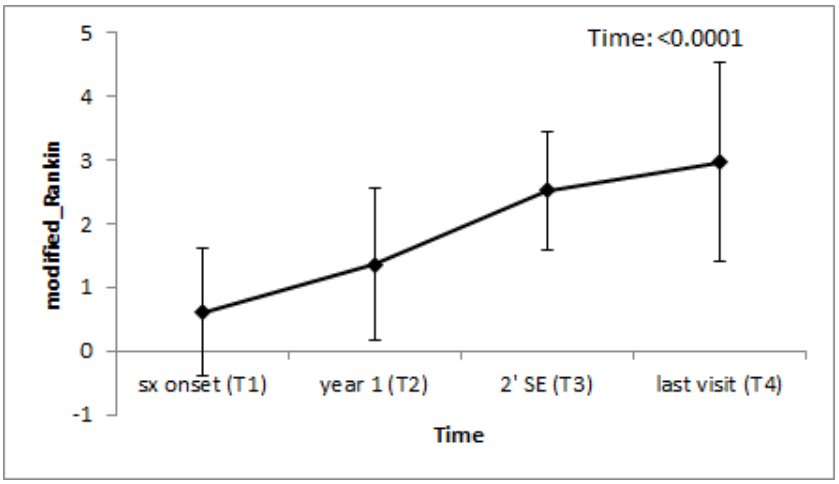


Figure 5. modified Rankin scale (mRS) scores with disease progression

Table 5 and Figures 6-1, 2, 3, 4, 5 show the NPMDS score trend with time (n=16). NPMDS was applied in subjects below age 18 years until the last follow up, and I calculated total scores for sections 1, 2 and 3, as well as selected questions ("NPMDS_neuromuscular") which matched with the modified version of

Neuromuscular_total for comparison. As seen, all 5 scores increased over time with statistical significance (all *P*<.0001, respectively), reflecting worsening function in these subjects.

Table 5. The Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) scores with disease progression

	NPMDS_ total (n=16)	**NPMDS_ section 1 (n=16)**	**NPMDS_ section 2 (n=16)**	**NPMDS_ section 3 (n=16)**	**NPMDS_ Neuro-muscular (n=16)**
Symptom onset (n=16)	11.0±7.4 (1-30)	2.0±3.1 (0-9)	4.3±2.8 (0-10)	3.9±3.7 (0-11)	7.0±2.9 (1-12)
Year 1 (n=16)	10.0±9.5 (1-36)	3.6±4.3 (0-16)	2.5±1.4 (0-5)	4.1±4.7 (0-16)	5.0±4.5 (1-17)
2nd stroke-like episode (n=14)	23.0±9.1 (8-41)	7.5±4.6 (2-17)	6.5±1.7 (4-10)	9.3±4.3 (1-16)	12.0±4.4 (3-19)
Last visit (n=16)	36.0±14.6 (12-63)	12.1±6.7 (2-21)	9.8±4.4 (3-19)	14.3±5.1 (4-23)	15.0±4.1 (7-23)
P-value	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*

**P*-value was calculated using linear mixed model.

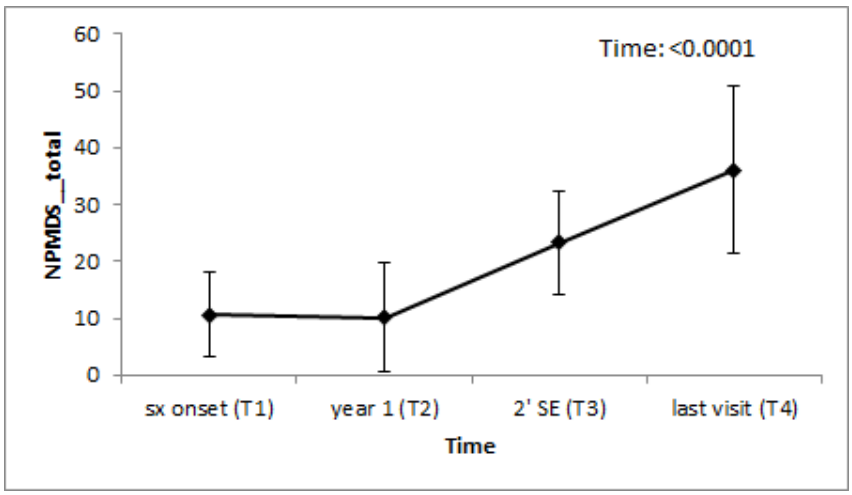


Figure 6-1. The Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) score_total with disease progression

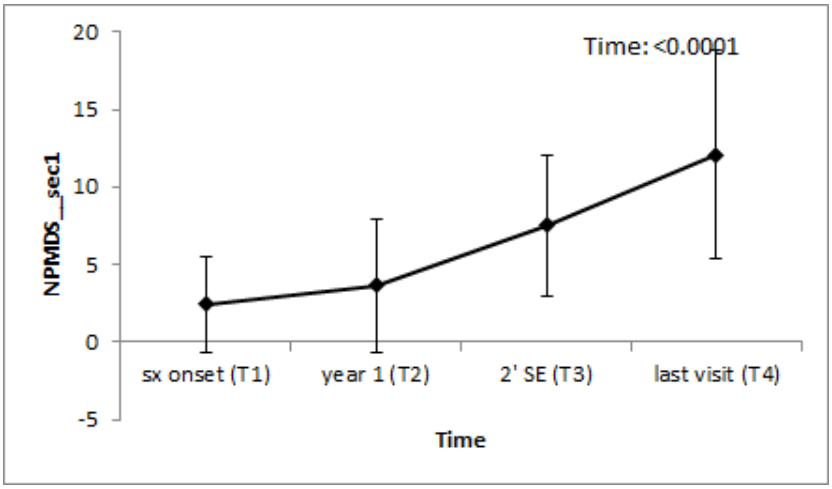


Figure 6-2. The Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) score_section 1 with disease progression

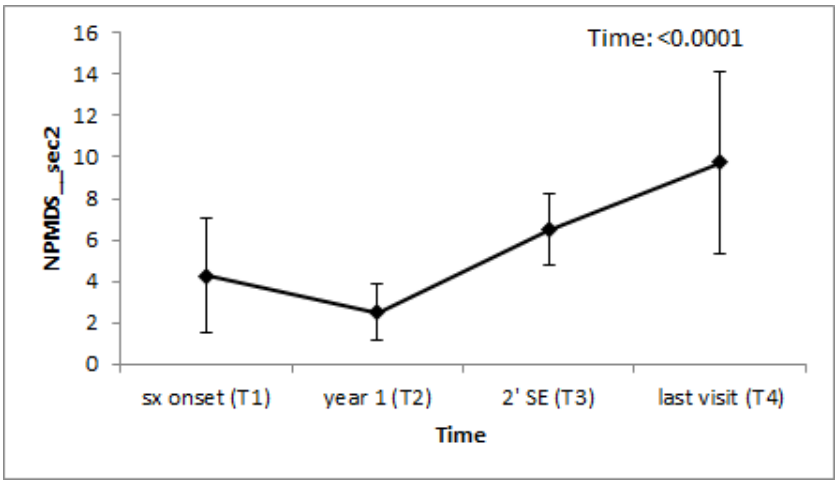


Figure 6-3. The Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) score_section 2 with disease progression

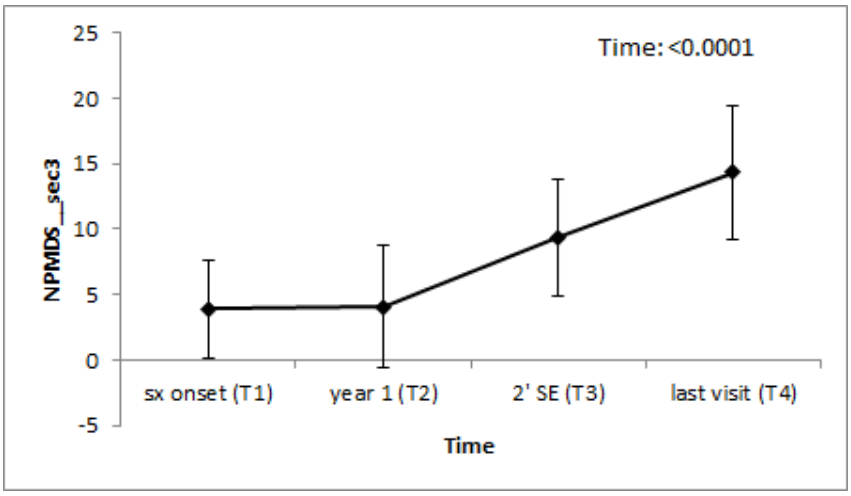


Figure 6-4. The Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) score_section 3 with disease progression

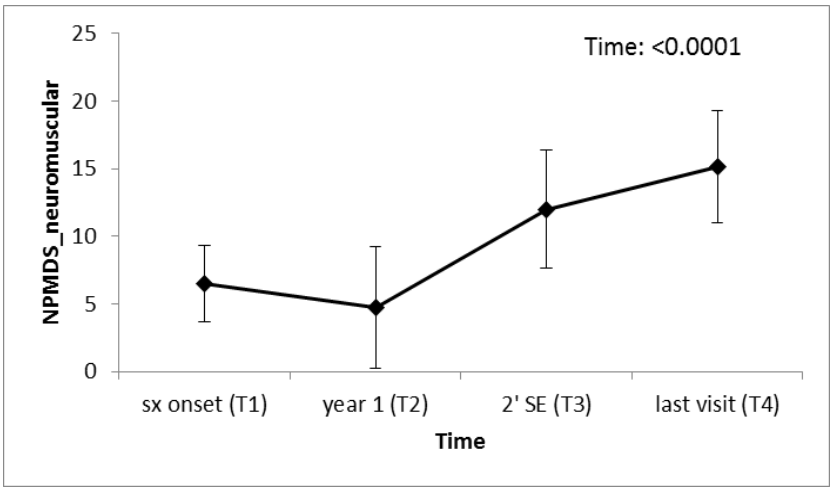


Figure 6-5. The Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) score_neuromuscular with disease progression

Table 6 and Figures 7-1, 2, 3, 4, 5 show NMDAS score trend with respect to changes with time (n=9). NPMDS was applied to subjects over age 18 years from diagnosis, and I calculated the total score, each section 1, 2 and 3, and selected questions (“NMDAS_neuromuscular”) that matched with the modified version of

Neuromuscular_total for comparison. As seen, all 5 scores increased over time with statistical significance (*P* values: 0.0081, 0.0092 0.0022, 0.0154 and <.0001, respectively), reflecting worsening function in these subjects.

Table 6. The Newcastle Mitochondrial Adult Disease Scale (NMDAS) scores with disease progression

	NMDAS_ total n=9)	**NMDAS_ section 1 (n=9)**	**NMDAS_ section 2 (n=9)**	**NMDAS_ section 3 (n=9)**	**NMDAS_ Neuro-muscular (n=9)**
Symptom onset (n=9)	12.0±6.2 (5-24)	2.4±2.1 (0-7)	7.4±3.5 (2-12)	2.0±2.3 (0-6)	8.0±4.2 (1-13)
Year 1 (n=9)	17.0±17.3 (5-58)	7.6±9.0 (1-27)	5.5±3.7 (2-14)	4.4±6.2 (0-17)	6.0±7.8 (1-24)
2nd stroke-like episode (n=7)	31.0±14.1 (16-54)	11.3±7.0 (4-22)	11.3±3.4 (7-16)	9.0±4.9 (3-16)	12.0±9.2 (0-24)
Last visit (n=9)	40.0±24.6 (11-86)	17.6±13.0 (3-43)	11.1±5.9 (3-18)	12.0±7.9 (3-25)	16.0±9.9 (4-30)
P-value	0.0081*	0.0092*	0.0022*	0.0154*	<.0001*

**P*-value was calculated using linear mixed model.

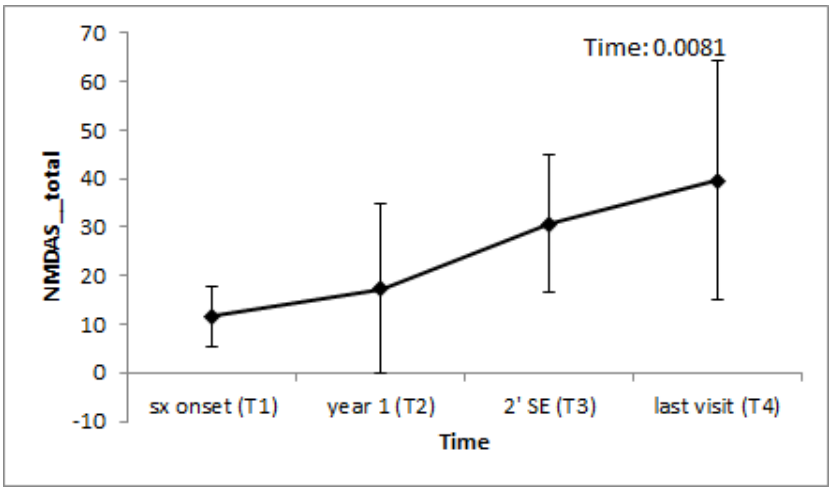


Figure 7-1. The Newcastle Mitochondrial Adult Disease Scale (NMDAS) score_total with disease progression

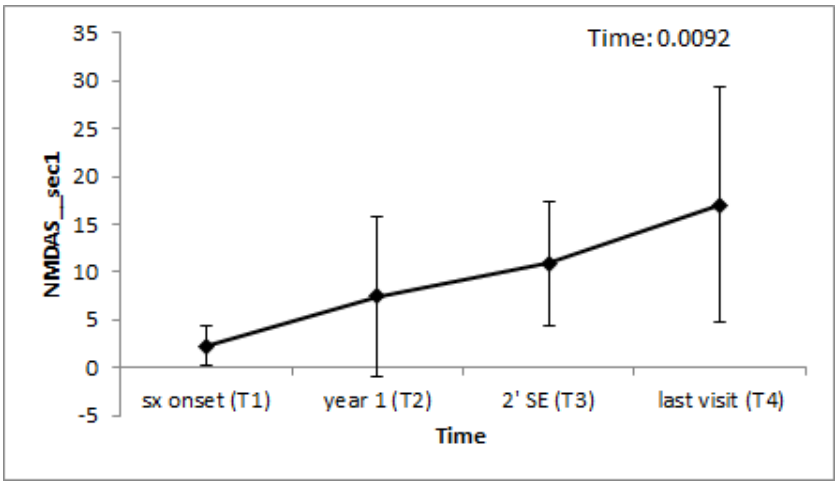


Figure 7-2. The Newcastle Mitochondrial Adult Disease Scale (NMDAS) score_section 1 with disease progression

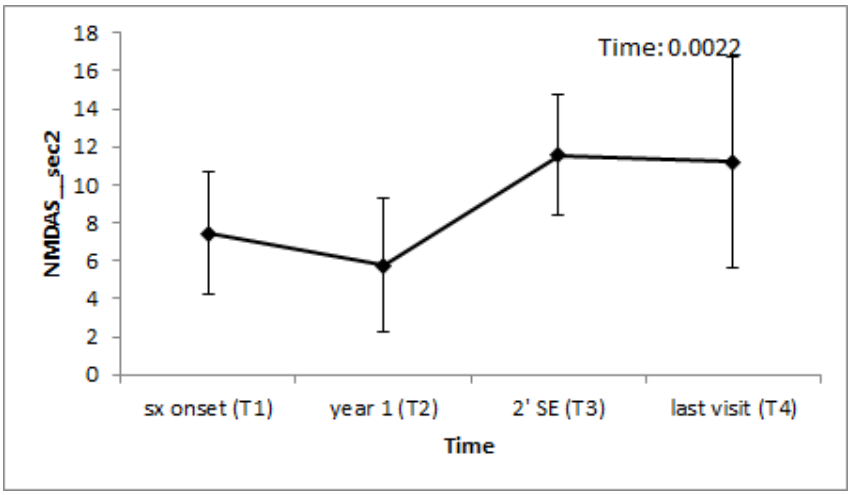


Figure 7-3. The Newcastle Mitochondrial Adult Disease Scale (NMDAS) score_section 2 with disease progression

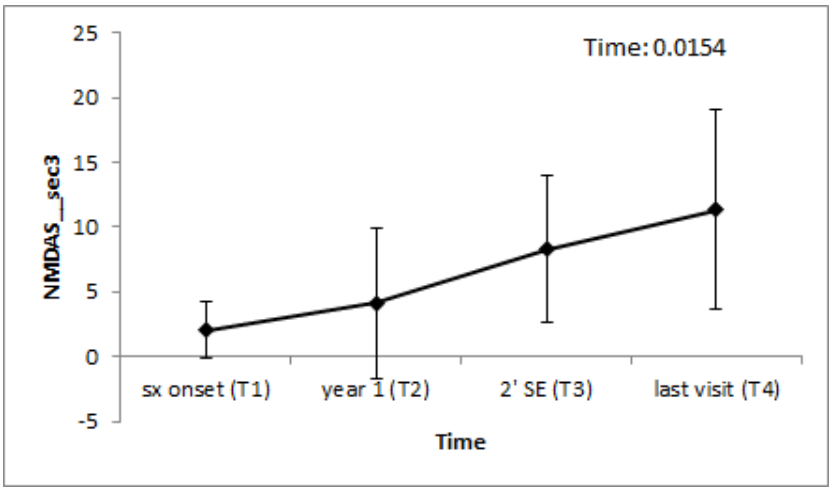


Figure 7-4. The Newcastle Mitochondrial Adult Disease Scale (NMDAS) score_section 3 with disease progression

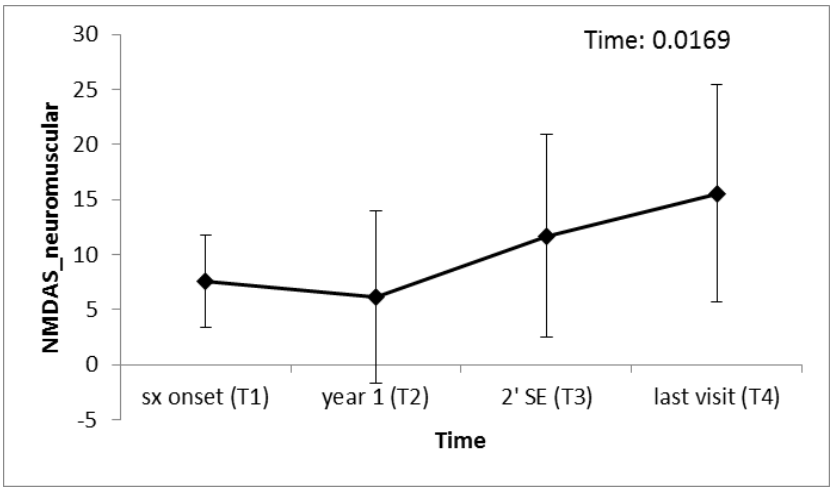


Figure 7-5. The Newcastle Mitochondrial Adult Disease Scale (NMDAS) score_neuromuscular with disease progression

Table 7 and Figure 8 shows Function_total score trend with time in MELAS. The Function_total score evaluated the motor, verbal, and social function, and the total score ranged from 0 to 7, and a higher score indicating worse function. The Funtion_total score increased over time with statistical significance (P<.0001), reflecting worsening function with disease progression.

Table 7. Function_total scale scores with disease progression

	Function_total score (N=25)
Symptom onset (n=25)	1.0±1.1 (0-3)
Year 1 (n=25)	2.0±2.3 (0-7)
2nd stroke-like episode (n=21)	3.0±1.7 (0-7)
Last visit (n=25)	4.0±2.1 (1-7)
P-value	<.0001*

**P*-value was calculated using linear mixed model.

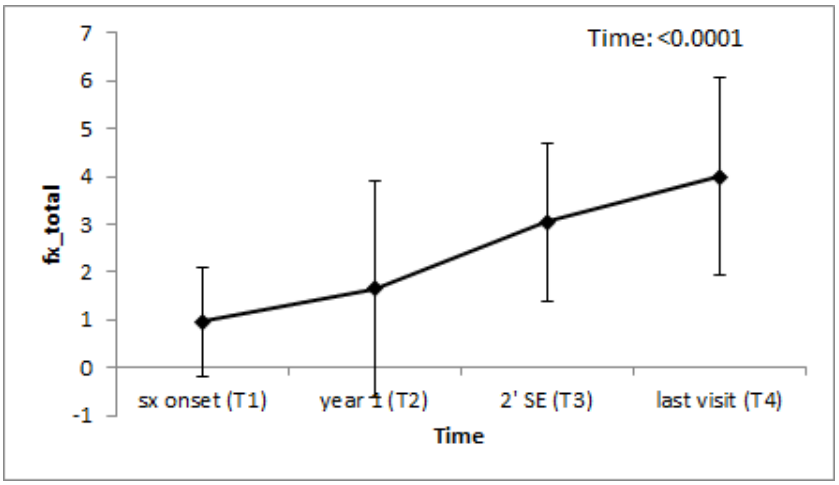


Figure 8. Function_total scale scores with disease progression

Table 8 and Figure 9 show Neuromuscular_total score trend with respect to changes with time in MELAS. The Neuromuscular_total score evaluated 14 different neurologic and muscular manifestations associated with MELAS and the total score ranged from 0 to 15, and a higher score indicated worse function. The Neuromuscular_total score increased over time with statistical significance (P<.0001), reflecting worsening function with disease progression.

Table 8. Neuromuscular_total scale scores with disease progression

	Neuromuscular_total scale score (N=25)
Symptom onset (n=25)	4.0±1.6 (1-7)
Year 1 (n=25)	3.0±2.1 (1-10)
2nd stroke-like episode (n=21)	7.0±2.4 (3-12)
Last visit (n=25)	9.0±2.9 (4-15)
P-value	<.0001*

**P*-value was calculated using linear mixed model.

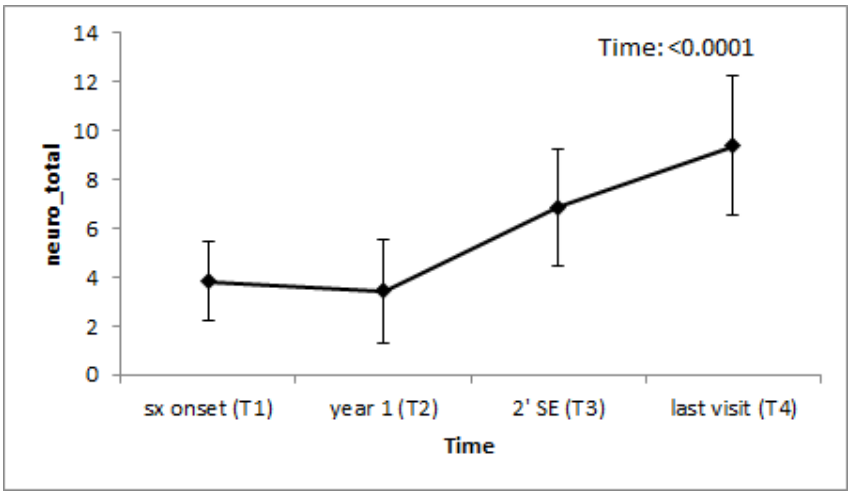


Figure 9. Neuromuscular_total scale scores with disease progression

Table 9 and Figure 10 show Non-neuromuscular_total score trend with respect to changes with times in MELAS. The Non-neuromuscular_total score evaluated the involvement of 11 systemic organs apart from the neurologic and muscular manifestations associated with MELAS and the total score ranged from 0 to 15, and a higher score indicated worse function. The Non-neuromuscular_total score increased over time with statistical significance (P<.0001), reflecting worsening function with disease progression.

Table 9. Non-neuromuscular_total scale scores with disease progression

	Non-neuromuscular_total scale score (N=25)
Symptom onset (n=25)	1.0±1.1 (0-5)
Year 1 (n=25)	2.0±1.8 (0-6)
2nd stroke-like episode (n=21)	4.0±1.7 (1-7)
Last visit (n=25)	7.0±3.3 (1-15)
P-value	<.0001*

**P*-value was calculated using linear mixed model.

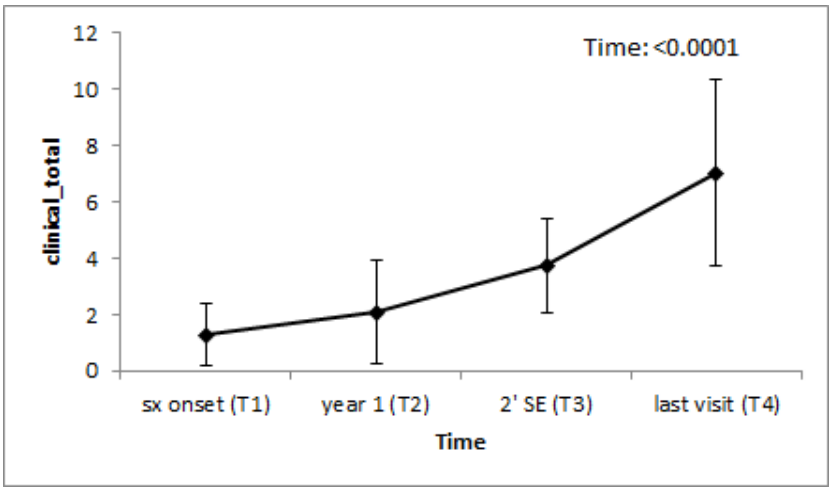


Figure 10. Non-neuromuscular_total scale scores with disease progression

5. Correlation between blood A3243G mutant load and clinical and diagnostic variables

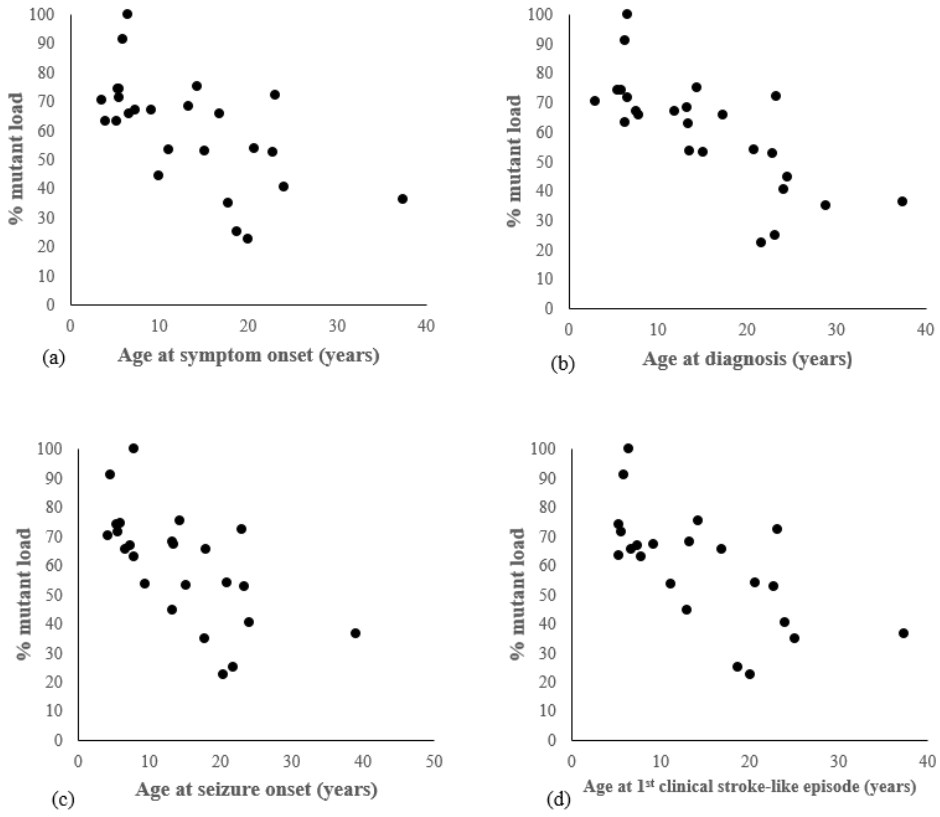
Table 10 and Figure 11 show correlation between blood A3243G mutant load and clinical/ diagnostic variables. As summarized, higher blood mutant load at genetic diagnosis showed inverse correlation with statistical significance with age at symptom onset, age at diagnosis, age at 1st clinical stroke-like episode, age at 1st MRI positive stroke-like episode, and age at seizure onset (*P*-values all <.0001, respectively). However, the level of blood mutant load did not correlate with times of recurrent stroke-like episode. The level of blood mutant load positively correlated with statistical significance with maximal absolute value of serum lactate (r=0.3346, *P*=0.0007), and with sum of abnormal findings (presence of lactate peak, decreased NAA and increased choline peak) in MRS (r=0.2917, *P*=0.0032) but not with sum of abnormal findings in MRI.

Table 10. Correlation between blood A3243G mutant load and clinical and diagnostic variables

Blood A3243G mutant load	r	P-value

Age at symptom onset	-0.6132	<.0001*
Age at diagnosis	-0.7436	<.0001*
Age at seizure onset	-0.646	<.0001*
Age at 1st clinical stroke-like episode	-0.6489	<.0001*
Age at 1st MRI positive stroke-like episode	-0.7619	<.0001*
Times of recurrent stroke-like episode	0.0212	0.834
Maximal absolute value of serum lactate	0.3346	0.0007*
Sum of abnormal findings in MRI	0.154	0.126
Sum of abnormal findings in MRS	0.2917	0.0032*

**P*-value was calculated using the Pearson correlation



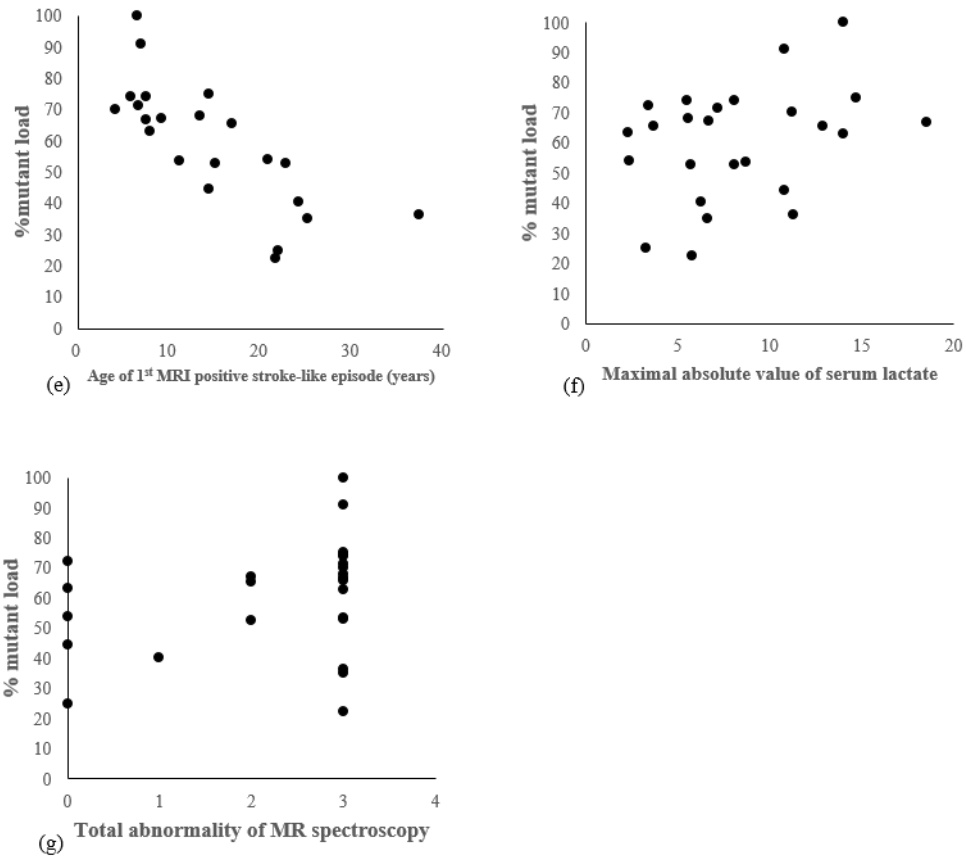


Figure 11. Correlation between blood A3243G mutant load and clinical and diagnostic variables

(a) Age at symptom onset
(b) Age at diagnosis
(c) Age at seizure onset
(d) Age at 1st clinical stroke-like episode
(e) Age at 1st MRI positive stroke-like episode
(f) Maximal absolute value of serum lactate
(g) Sum of abnormal findings in MRS

6. Correlation between blood A3243G mutant load and functional scale scores according to disease progression

Table 11 and Figure 12 show correlation between blood mutant load and mRS scale scores according to disease progression at 4 different time points. There was no statistically significant correlation at any of the 4 time points.

Table 11. Correlation between blood mutant load and modified Rankin scale (mRS) scores according to disease progression

Blood A3243G mutant load and mRS	**R**	***P*-value**
Total	0.1106	0.2835
Symptom onset	0.3266	0.111
Year 1	-0.0335	0.8738
2nd stroke-like episode	-0.2168	0.3451
Last visit	0.3015	0.2564

**P*-value was calculated using the Pearson correlation

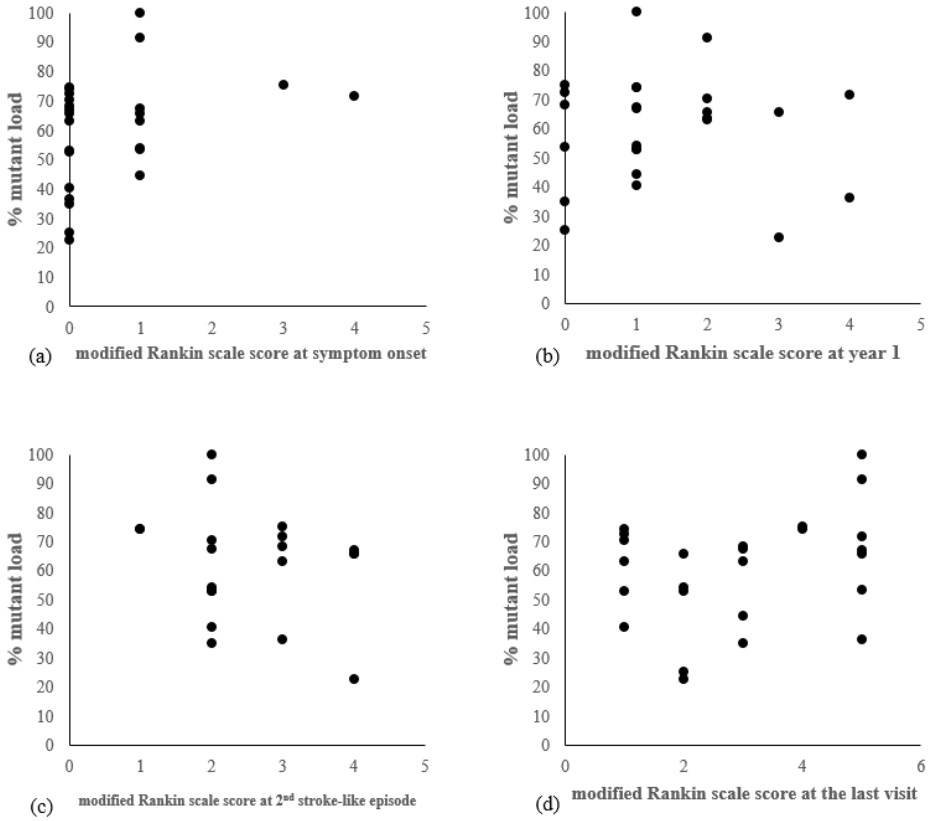


Figure 12. Correlation between blood mutant load and modified Rankin scale score according to disease progression

(a) At symptom onset
(b) At year 1
(c) At 2nd stroke-like episode
(d) At the last visit

Table 12 and Figure 13 show correlation between blood mutant load and NPMDS scale scores (n=16) according to disease progression at 4 different time points. There was no statistically significant correlation at any of the 4 time points.

Table 12. Correlation between blood mutant load and NPMDS scale scores according to disease progression (N=16)

Blood mutant load	NPMDS_total		NPMDS_neuromuscular	
	R	*P*-value	R	*P*-value
Total	0.987	0.454	0.1091	0.3988
Symptom onset	0.2785	0.2963	0.2283	0.3951
Year 1	0.0081	0.9764	0.0291	0.9149
2nd stroke-like episode	-0.1718	0.5569	0.0099	0.9732
Last visit	0.3015	0.2564	0.3089	0.2445

**P*-value was calculated using the Pearson correlation

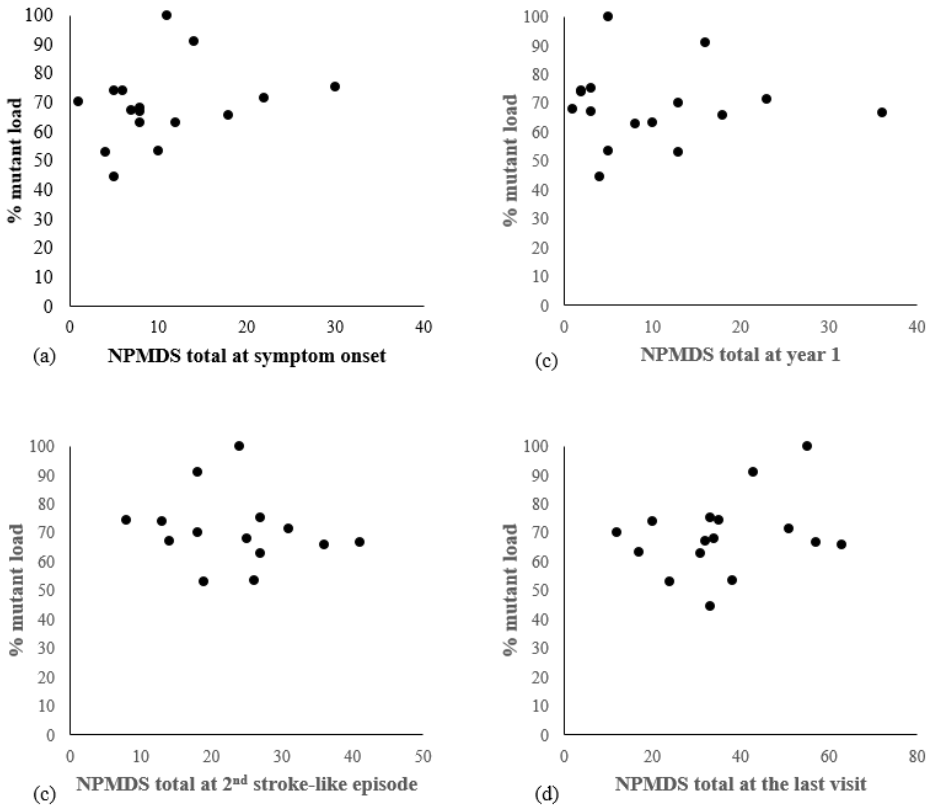


Figure 13. Correlation between blood mutant load and NPMDS_total scale score according to disease progression

(a) At symptom onset

(b) At year 1

(c) At 2nd stroke-like episode

(d) At the last visit

Table 13 and Figure 14 4 show correlation between blood mutant load and NMDAS scale scores (n=9) according to disease progression at 4 different time points. There was no statistically significant correlation at any of the 4 time points.

Table 13. Correlation between blood mutant load and NMDAS scale scores according to disease progression (N=9)

Blood mutant load	**NMDAS_total**		**NMDAS_neuromuscular**	
	R	***P*-value**	**R**	***P*-value**
Total	-0.0831	0.6405	-0.0948	0.5824
Symptom onset	0.1749	0.6527	0.1586	0.6836
Year 1	0.0873	0.8233	-0.0149	0.9696
2nd stroke-like episode	0.1964	0.6729	-0.0626	0.8728
Last visit	-0.4176	0.2634	-0.3418	0.3680

**P*-value was calculated using the Pearson correlation

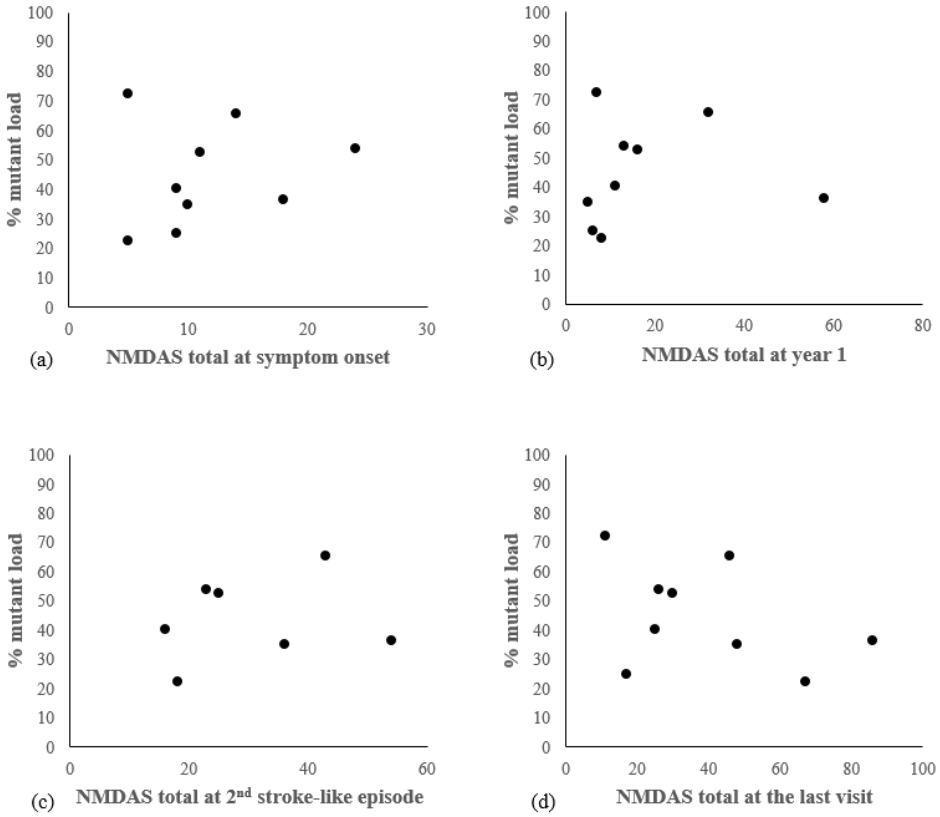


Figure 14. Correlation between blood mutant load and NMDAS_total scale score according to disease progression

(a) At symptom onset

(b) At year 1

(c) At 2nd stroke-like episode

(d) At the last visit

Table 14 and Figure 15 show correlation between blood mutant load and Function_total scale scores (n=25) according to disease progression at 4 different time points. Blood mutant load at genetic diagnosis positively correlated with Function_total at symptom onset with statistical significance (r=0.5476, *P*=0.0046) but not at different time points.

Table 14. Correlation between blood mutant load and Function_total scale scores according to disease progression (n=25)

Blood mutant load and Function_total	R	*P*-value
Total	0.1999	0.0509
Symptom onset	0.5476	0.0046*
Year 1	0.2042	0.3276
2nd stroke-like episode	-0.0655	0.0778
Last visit	0.3103	0.1312

**P*-value was calculated using the Pearson correlation

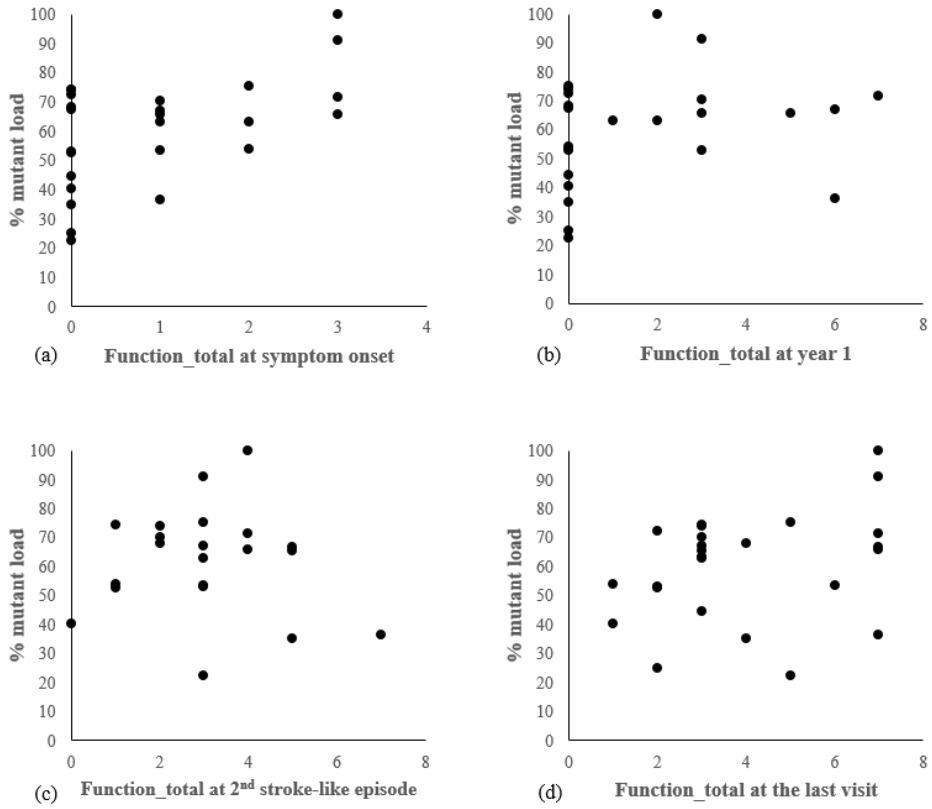


Figure 15. Correlation between blood mutant load and Function_total scale score according to disease progression

(a) At symptom onset

(b) At year 1

(c) At 2nd stroke-like episode

(d) At the last visit

Table 15 and Figure 16 show correlation between blood mutant load and Neuromuscular_total scale scores (n=25) according to disease progression at 4 different time points. Blood mutant load at genetic diagnosis positively correlated with Neuromuscular_total at the last visit with statistical significance (r=0.4418, *P*=0.027) but not at different time points.

Table 15. Correlation between blood mutant load and Neuromuscular_total scale scores according to disease progression (N=25)

Blood mutant load and Neuromuscular_total	**R**	***P*-value**
Total	0.0879	0.3946
Symptom onset	-0.0387	0.8541
Year 1	-0.1117	0.5951
2nd stroke-like episode	0.0482	0.8358
Last visit	0.4418	0.027*

**P*-value was calculated using the Pearson correlation

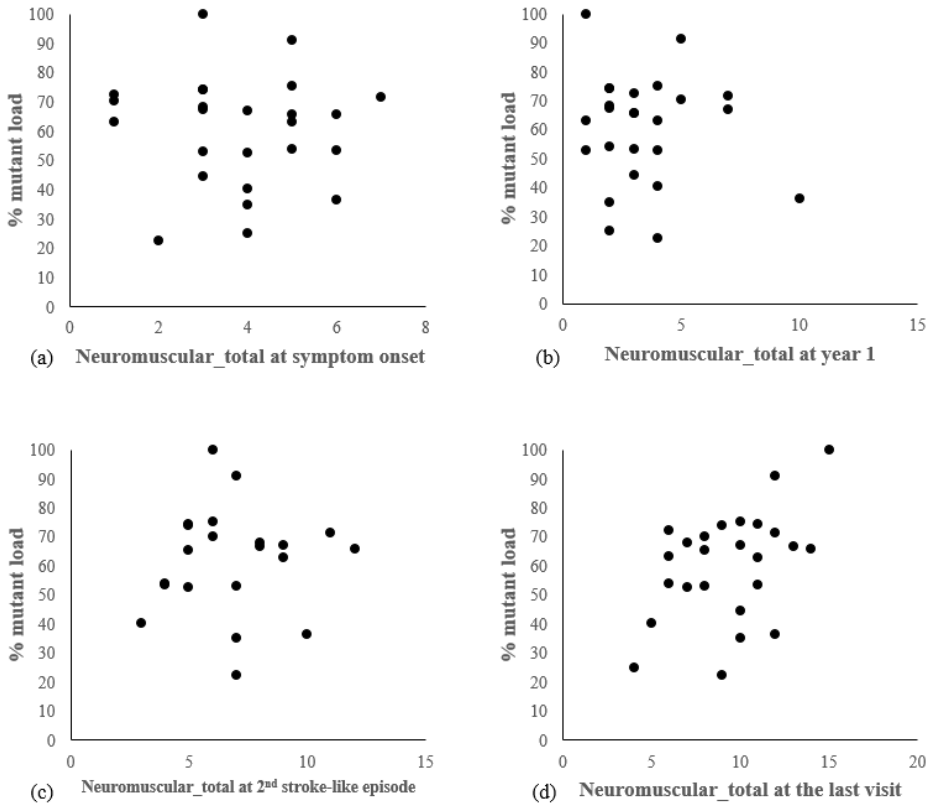


Figure 16. Correlation between blood mutant load and Neuromuscular_total scale score according to disease progression

(a) At symptom onset
(b) At year 1
(c) At 2nd stroke-like episode
(d) At the last visit

Table 16 and Figure 17 show correlation between blood mutant load and Non-neuromuscular scale scores (n=25) according to disease progression at 4 different time points. There was no statistically significant correlation at any of the 4 time points.

Table 16. Correlation between blood mutant load and Non-neuromuscular_total scale

scores according to disease progression (N=25)

Blood mutant load and Non-neuromuscular_total	**R**	***P*-value**
Total	0.0045	0.9651
Symptom onset	0.0153	0.9422
Year 1	-0.1524	0.4671
2nd stroke-like episode	-0.1511	0.5132
Last visit	0.1526	0.4665

**P*-value was calculated using the Pearson correlation

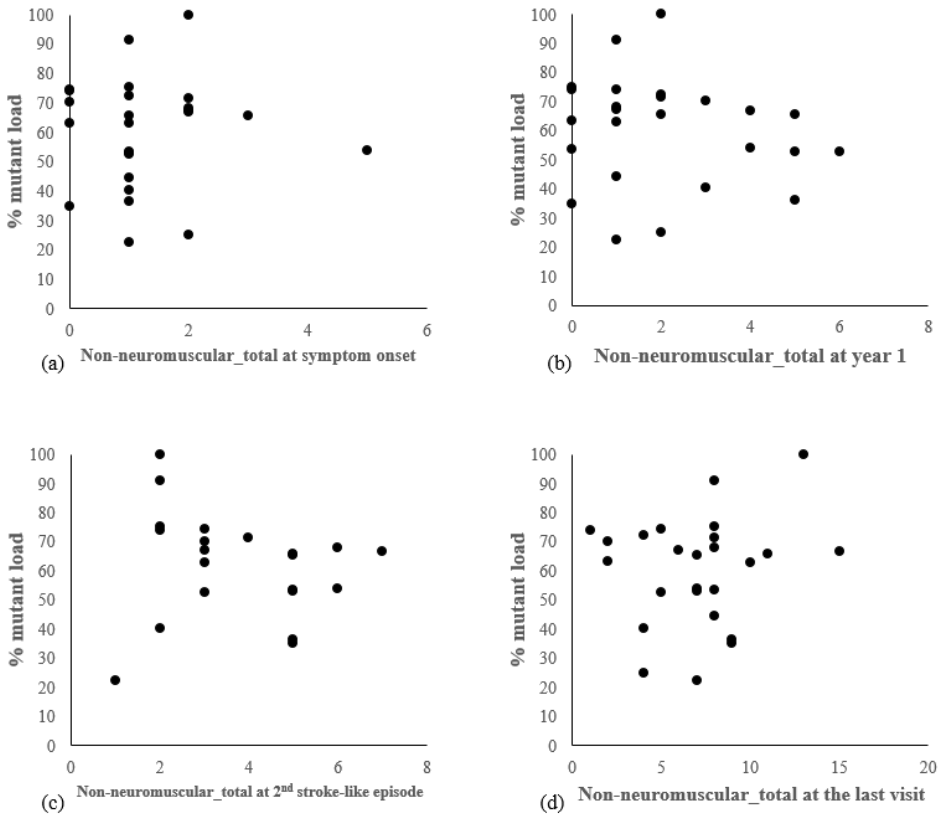


Figure 17. Correlation between blood mutant load and Non-neuromuscular_total scale score according to disease progression

(a) At symptom onset

(b) At year 1

(c) At 2nd stroke-like episode

(d) At the last visit

Table 17 shows comparison of correlation coefficients and P-values emphasizing neuromuscular function in 3 scales; NPMDS_neuromuscular (n=16), NMDAS_neuromuscular (n=9) and Neuromuscular_total (n=25). Blood mutant load at genetic diagnosis only correlated positively with Neuromuscular_total at the last visit (r=0.4418, *P*=0.027), indicating that higher blood mutant load correlated with worse neuromuscular function at the last visit.

Table 17. Correlation between blood mutant load and functional scale scores according to disease progression; neuromuscular aspect

	NPMDS_ neuromuscular (n=16)		**NMDAS_ neuromuscular (n=9)**		**Neuromuscular _total (n=25)**	
	R	***P*-value**	**R**	***P*-value**	**R**	***P*-value**
Total	0.1091	0.3988	-0.0948	0.5824	0.0879	0.3946
Symptom onset	0.2283	0.3951	0.1586	0.6836	-0.0387	0.8541
Year 1	0.0291	0.9149	-0.0149	0.9696	-0.1117	0.5951
2nd stroke-like episode	0.0099	0.9732	-0.0626	0.8728	0.0482	0.8358
Last visit	0.3089	0.2445	-0.3418	0.3680	0.4418	0.027*

**P*-value was calculated using the Pearson correlation

7. Correlation between blood A3243G mutant load and Δfunctional scale scores according to disease progression

As above mentioned, I could not find specific correlation between blood mutant load at genetic diagnosis and functional scale scores according to disease progression at each time point. Thus, I further examined if there was a correlation between the

blood mutant load and Δfunctional scale scores, and in other words, slope of functional scale score change between each time point (see Figure 18).

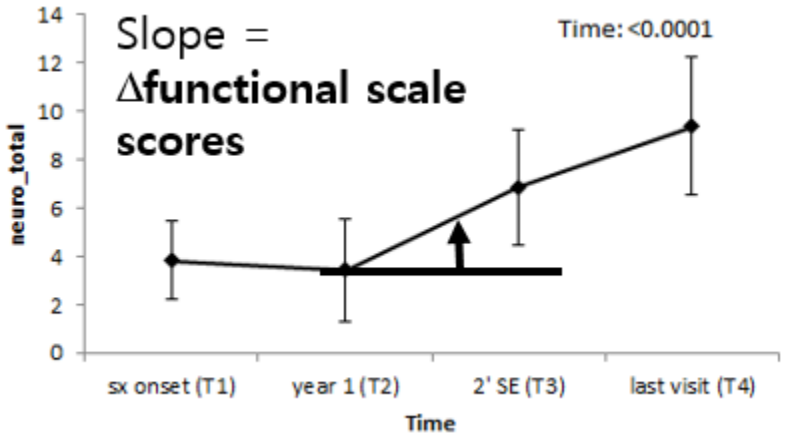


Figure 18. Slope of functional scale score change

Table 18 summarizes the correlation between blood mutant load at genetic diagnosis and Δfunctional scale scores according to disease progression. There was no correlation between blood mutant load at genetic diagnosis and Δfunctional scale scores with time in 3 different ways (symptom onset – year 1- 2nd stroke-like episode – last visit vs. symptom onset – year 1 – last visit vs. symptom onset – 2nd stroke-like episode – last visit), respectively in all functional scales apart from ΔNeuromuscular_total (r=0.5073, *P*=0.0096 vs. r=0.532, *P*=0.0062 vs. r=0.4698, *P*=0.0178).

Table 18. Correlation between blood mutant load at genetic diagnosis and Δ functional scale scores according to disease progression

Correlation with blood mutant load at genetic diagnosis	Symptom onset – year 1 - 2nd stroke-like episode – last visit	Symptom onset – year 1 – last visit (n=25)	Symptom onset - 2nd stroke-like episode – last visit (n=21)

	(n=21)					
	R	***P*-value**	**R**	***P*-value**	**R**	***P*-value**
ΔModified Rankin score (n=25)	-0.0269	0.8986	0.0024	0.9908	-0.0451	0.8305
ΔNPMDS_total (n=16)	0.1593	0.5557	0.2267	0.3985	0.1099	0.6853
ΔNPMDS_ neuromuscular (n=16)	0.2266	0.3987	0.227	0.398	0.1444	0.5936
ΔNMDAS_total (n=9)	-0.4878	0.1828	-0.5147	0.1562	-0.4376	0.2388
ΔNMDAS_ neuromuscular (n=9)	-0.4151	0.2666	-0.4586	0.2144	-0.3979	0.2889
ΔFuntion_total (n=25)	-0.0672	0.7495	0.0326	0.8771	-0.0686	0.7445
ΔNeuromuscular_ total (n=25)	0.5073	0.0096*	0.532	0.0062*	0.4698	0.0178*
ΔNon-neuromuscular _total (n=25)	0.1527	0.4663	0.1788	0.3924	0.1303	0.5348

**P*-value was calculated using the Pearson correlation

Figure 19 shows spaghetti and scatter plots of the correlation between blood mutant load and Δfunctional scale scores according to disease progression, from symptom onset to the last visit.

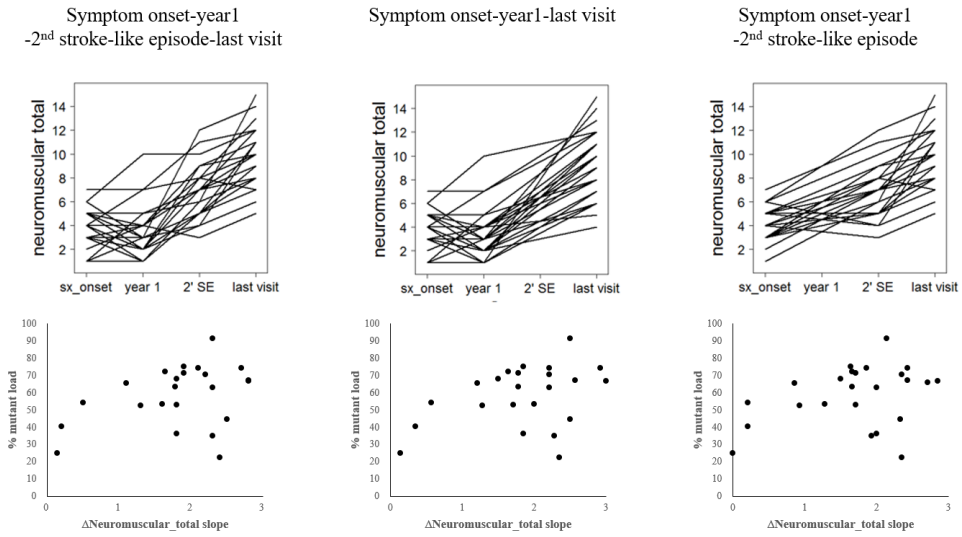


Figure 19. Spaghetti and scatter plots of the correlation between blood mutant load and Δfunctional scale scores according to disease progression

8. Correlation between blood A3243G mutant load and Δfunctional scale scores according to disease progression per month

Table 19 summarizes the correlation between blood mutant load at genetic diagnosis and Δfunctional scale scores according to disease progression per month. There was no correlation between blood mutant load at genetic diagnosis and Δ functional scale scores with time in 3 different ways (symptom onset – year 1- 2nd stroke-like episode – last visit vs. symptom onset – year 1 – last visit vs. symptom onset – 2nd stroke-like episode – last visit), respectively in all functional scales apart from ΔNeuromuscular_total (r=0.4284, *P*=0.0326 vs. r=0.428, *P*=0.0328 vs. r=0.4012, *P*=0.0468).

Table 19. Correlation between blood mutant load at genetic diagnosis and Δ functional scale scores according to disease progression per month

Correlation with blood	Symptom onset	Symptom onset	Symptom onset

mutant load at genetic diagnosis	– year 1 - 2nd stroke-like episode – last visit		– year 1 – last visit		- 2nd stroke-like episode – last visit	
	R	***P*-value**	**R**	***P*-value**	**R**	***P*-value**
ΔModified Rankin score (n=25)	0.1204	0.5666	0.112	0.594	0.106	0.6142
ΔNPMDS_total (n=16)	0.147	0.5869	0.1693	0.5308	0.1256	0.6431
ΔNPMDS_neuromuscular (n=16)	0.0212	0.938	0.0584	0.8299	0.0029	0.9915
ΔNMDAS_total (n=9)	-0.2173	0.5744	-0.205	0.5967	-0.2055	0.5959
ΔNMDAS_neuromuscular (n=9)	-0.1508	0.6986	-0.0125	0.9745	-0.0364	0.926
ΔFuntion_total (n=25)	0.1624	0.4381	0.176	0.4002	0.1499	0.4744
ΔNeuromuscular_total (n=25)	0.4284	0.0326*	0.428	0.0328*	0.4012	0.0468*
ΔNon-neuromuscular_total (n=25)	0.2529	0.2226	0.246	0.2358	0.2307	0.2672

**P*-value was calculated using the Pearson correlation

Figure 20 shows spaghetti and scatter plots of the correlation between blood mutant load and Δfunctional scale scores according to disease progression per month, from symptom onset to the last visit.

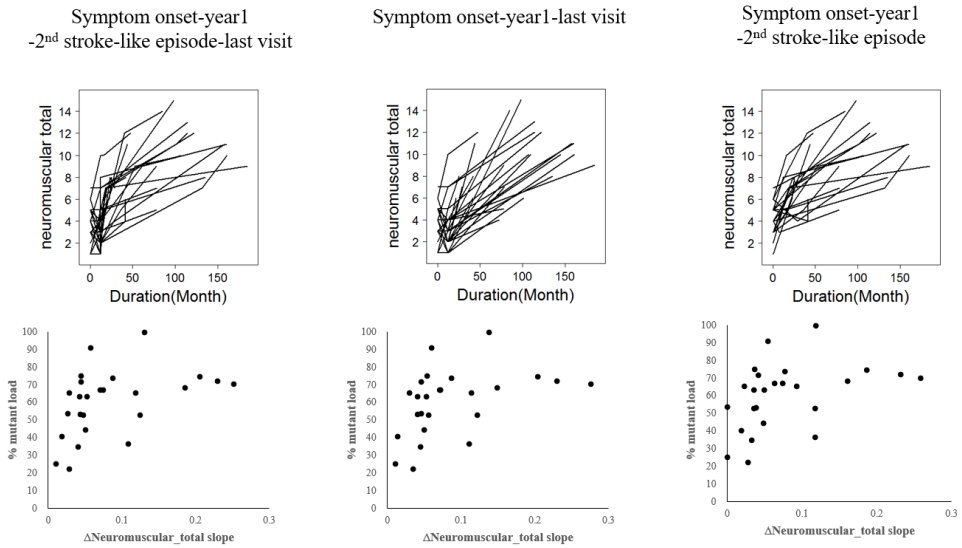


Figure 20. Spaghetti and scatter plots of the correlation between blood mutant load and Δfunctional scale scores according to disease progression per month

IV. DISCUSSION

Since the first description of MELAS according to its specific clinical features became available in literature in 1984[2], the most common genetic mutation of A to G transition at nucleotide position 3243 of mitochondrial DNA MT-TL1 gene encoding $tRNA^{LEU(UUR)}$ has been found by several investigators[8-12]. About 80% of MELAS syndrome patient harbor this specific mutation however on the contrary, not all carriers of this mutation suffers with MELAS. The A3243G mutation carriers can manifest in an extremely heterogenous way, from asymptomatic carriers (not yet symptomatic) to patients with maternally inherited diabetes and deafness syndrome[41], hypertrophic cardiomyopathy[3,4], and fully symptomatic MELAS, which prompted characterization of this specific mutation and accordant genotype-phenotype correlation studies.

The mitochondrial DNA exists literally in all types of tissues and cells apart from red blood cells, and defective mtDNA results in ineffective oxidative phosphorylation

and improper energy production. This leads to energy failure in different organs; however, the level of organ failure varies, making clinical spectrum even more diverse. MELAS was characterized by three distinct clinical criteria in 1992, which includes stroke-like episodes before the age of 40, encephalopathy characterized by seizures and/or dementia, and mitochondrial myopathy evidenced by lactic acidosis and/or ragged-red fibers in the muscle biopsy[1, 3, 5], which has been further expanded and updated[15] since then. In symptomatic MELAS patients, usually the clinical trajectory deteriorates over time. This ongoing degeneration results not only because of the involvement of various organs but also the neurologic and muscular symptoms, accordant disabilities in daily living, and restrictions in social, educational, and vocational life. Recurrent stroke-like episodes are one of the principal clinical features in MELAS patients, and repetitive cortical damage and additional neurodegenerative process contributes to further debilitation[3]. Other neurologic manifestations including developmental delay, dementia, epilepsy and associated status epilepticus events, peripheral neuropathy, myopathy and ataxia can also add to worsening neuromuscular function. Considering this, the exact understanding of natural history and declining pattern in these patients and investigation of correlative biomarkers to predict disease progression is important.

As A3243G pathogenic mutation is the most common mutation in MELAS patients, its detection has been available from the discovery of this mutation and has progressed over time with new techniques including NGS method. The mitochondrial DNA mutation has special features unlike the nuclear DNA mutation, so called heteroplasmy. Heteroplasmy characterizes and further complicates the diseases caused by mtDNA mutation as the normal and mutant mtDNAs are mixed within the same cell and their ratio varies among tissues and organs[33]. It is known that the level of heteroplasmy, or mutant load can vary between the offsprings from the same mother, and the level of mutant load in maternal DNA can affect the outcome of pregnancy and risks of having affected offspring, suggesting the effect of higher blood mutant load on severe clinical phenotype[17,18] In the genetic context, confirmation of the pathogenic mtDNA mutation and characterization of mutation

itself, and detection and analysis of mutant load have been considered important for genotype-phenotype correlation[16-19, 42-45].

Several methods including real-time polymerase chain reaction (RT-PCR)[22], pyrosequencing[31, 32] and NGS have been employed to calculate the mtDNA mutant load[23-30]. Compared to the other methods, NGS has the advantage of providing millions of DNA reads in a single run at a low cost[23]. NGS is also proficient in diagnosing mitochondrial disease especially in terms of clinical and genetic heterogeneity, when the clinical manifestations do not meet the criteria for certain mitochondria syndrome[30], considering the recommendations of the American College of Medical Genetics and Genomics which states that whole exome sequencing and genome sequencing should be considered in the clinical diagnostic assessment of patients with high degree of genetic heterogeneity[24]. Using NGS, the pathogenic mutation in mtDNA can be confirmed and the level of mutant load can by analyzed simultaneously. In my specific study patients could have been easily diagnosed with Sanger sequencing as these subjects met clinical criteria for MELAS syndrome. Yet as aforementioned, because of extreme clinical and genetic heterogeneity, A3243G mutation can present in various forms from the asymptomatic carriers to fully symptomatic MELAS patients[12, 14]; on the contrary, symptomatic MELAS patients can harbor other mtDNA mutation apart from the most common A3243G mutation[13]. In that context, using NGS primarily to diagnose mitochondrial phenotype with consequential confirmation with Sanger sequencing would decrease burden of repetitive separate sequencing and associated trial and error, in addition to the advantages of being less time consuming and cost effective. In addition, the ability of NGS to analyze and accurately detect even the low-level heteroplasmy[23, 25-29] also justified the use of NGS method for this study to obtain data for pathogenic A3243G mutation and the level of mutant load simultaneously.

Mutant load was previously studied in variable samples including the skeletal muscle, blood leukocyte, urine epithelial cells and buccal mucosa. It has been said that in the post-mitotic tissue such as the skeletal muscle samples, mutant load is consistently higher when compared to the other samples[46-48]. However, obtaining the

muscle samples several times for research purpose is not possible, and the muscle biopsy is invasive and burdensome even for the therapeutic monitoring purpose. Therefore, easier and less invasive sampling method of obtaining blood leukocytes, urinary epithelial cells, and buccal mucosa was largely studied in A3243G positive subjects[49]. It has been observed by the other researchers that urine epithelial cells are superior in obtaining consistent levels of mutant load over blood leukocytes [49-53]. The laboratory methodology of obtaining blood leukocytes and confirmation of genetic mutation and calculation of mutant load may not be particularly unique, given that there are abundant previous studies that have used this method. Yet in my study, I opted to study mtDNA A3243G mutant load from the blood leukocyte for the following reasons : 1) obtaining mutant load from urine epithelial cell as a routine procedure was not performed at the beginning of my study inclusion period; 2) obtaining mutant load from the same blood leukocyte sample at the genetic diagnosis would guarantee consistent findings than using any other type of samples; 3) I hypothesized that the blood mutant load obtained at the genetic diagnosis would reflect disease burden early in the disease course for each MELAS patient.

Several studies have investigated the mutant load and effect of age, and consistently showed that the level of mutant load decreases with age or disease progression[19, 20, 48, 53-55]. In my study, mean mtDNA A3243G mutant load at the time of genetic diagnosis was 60.2±18.8% (range: 22.5-100) at the mean age of 15.2±8.8 years (range: 3.0-37.7). As shown in Figure 4, there was a significant inverse correlation between age and mutant load (r=-0.737, *P*=.000 by the Pearson correlation), which is consistent with previously published researches[19-21]. Younger patients, or patients who were younger at the time of genetic diagnosis had higher blood mutant load. However, my observation has the limitation of obtaining single mutant load measurement from a single subject, restricting further investigation of longitudinal changes within the same subject or investigation of effect of age adjusted mutant load on functional outcome. Mehrazin et al. calculated the blood mutant load via RT-PCR method in 17 fully symptomatic MELAS patients over a mean period of 7 years with resultant average mutant load percentage decreases over time (estimated

slope of -0.534 with *P*-value of 0.0085)[20]. Recently, Grady et al. reported that the blood mutant load level calculated by pyrosequencing method negatively correlated with age, with a compound decline of -2.3%/ year over a period 4 or more years in 195 symptomatic MELAS patients[19], necessitating future research with repetitive measurements of mutant load according to disease progression and clinical deterioration events involving subjects selected in the current study.

Objective evaluation of functional outcome in mitochondrial disease patients has been pursued in several studies[57]. As well known, mitochondrial disease comprises of extremely heterogenous clinical manifestations and involvement of various organs of varying severity, making comprehensive evaluation even more difficult. Some researchers applied previously known scales in their studies, whereas others tried to develop new scales to meet the needs of this special population[57]. The Newcastle Pediatric Mitochondrial Disease Scale (NPMDS)[39] and Newcastle Mitochondrial Disease Adult Scale (NMDAS)[40] have been developed and validated for that particular purpose and are continually being used in studies to describe natural history, and in clinical trials to prove efficacy and safety of certain treatment regimens in various mitochondrial diseases[15, 43, 58-62]. These two scales have been also used in several previous studies to investigate the natural course of disease progression in MELAS. Other studies have applied different functional scales that have been either previously used for other disease categories and associated clinical trials[34-38, 42, 63] or newly developed for the study design[15]. The mRS was developed for use in clinical trials and follow up purposes in stroke patients, including young adult and children population[34-38] and has been used to evaluate the functional status and changes over time in MELAS related studies[42, 63-65]. Kaufmann et al. [21, 68] applied Karonofsky score and Columbia Neurological score to evaluate the functional status and timely changes in MELAS patients. A Japanese group used the JMDRS score to analyze clinical progress[15], which was not been validated by the time of the study but contents were similar to the validated scales of NMDAS and NPMDS[39 ,40]. In other studies, survival analysis by Kaplan-Meier method[42, 63], plasma lactate level[69], maximal oxygen uptake (VO_2max) and maximal workload[69], and frequency of certain clinical manifestations

such as myopathy, stroke-like episodes, diabetes mellitus[16, 17, 64, 66, 67], brain MRI changes[59] or cerebral lactic acidosis in brain MRS[21] had also been used to objectively evaluate functional outcome and changes in MELAS patients or wider population of A3243G mutation carriers. Some of these studies focused on investigating the natural history of MELAS whereas other studies investigated functional changes in relation to mtDNA mutant load from different samples.

In the current study, I applied all 6 functional scales to objectively evaluate the functional outcome in symptomatic MELAS patients and track their changes at 4 different time points. First, I used previously validated scales of mRS (n=25), NPMDS (n=16) and NMDAS (n=9), according to the age range during the study period. In addition, I designed modified and simplified versions of 3 functional scales for this study: Function_total (n=25), Neuromuscular_total (n=25), and Non-neuromuscular_total (n=25). Function_total was developed to cover the overall general functional status including physical, mental, educational/ vocational quality of life, and the ability to achieve daily requirements; in a comprehensive manner but also in less time. In comparison, mRS can be easily applied to stroke patients and has been validated for a long time in clinical trials, with narrow score ranges of 0 to 6 focusing on the disability from the stroke event itself. NPMDS and NMDAS had also been validated throughout numerous researches and widely used and have the advantage of comprehensive evaluation of the full spectrum of mitochondrial disease from current function to system specific involvement and clinical assessment. Nevertheless, NPMDS and NMDAS consist of large number of questions and wide score range for each question, which is time-consuming. In that context, Neuromuscular_total and Non-neuromuscular total were designed to simplify the score range for each question and focus on neuro-muscular (central nervous system and peripheral nervous system related manifestations) vs. non-neuromuscular (involvement of all other organ systems) that is easily applicable within shorter time, regardless of age range.

Previously known and validated functional scale scores of mRS (n=25), NPMDS (n=16) and NMDAS (n=9) increased with significance with disease progression,

reflecting worsening disease course (all *P*<.0001, respectively). This was also observed with Function_total (n=25), Neuromuscular_total (n=25) and Non-neuromuscular total (n=25) with statistical significance (all *P*<.0001, respectively) reflecting worsening disease course in these patients. From these data, I can infer that the symptomatic MELAS patients continues to decline in function with disease progression, which can be evaluated and tracked using previously known validated scales and my simplified versions of scales. Regular use of these functional scales in MELAS patients would enable better prediction of disease trajectory, and further research could be expanded to other mitochondrial syndromes or non-specified type of mitochondrial disease patients.

As the functional scales scores were verified for use, the correlation of these functional scales with mtDNA A3243G mutant load was investigated. Quantitative analysis of mtDNA mutant load and investigation in relation to the clinical phenotype has been studied by several researchers but no explicit conclusion has been reached in this regard[16-21, 49, 60, 63-65]. Chinnery et al. reported the correlation of mutant load from the skeletal muscle samples with higher frequency of recurrent stroke-like episodes, dementia, epilepsy and ataxia[16, 17], whereas de Laat et al. reported the relationship between urinary epithelial cell mutant load and certain clinical symptoms but with a relatively low correlation coefficient[18, 49]. Grady et al. reported that the mutant load variability in blood leukocyte is lower than any other samples and the blood mutant load shows an association with disease burden and progression using serial measurement and functional scale scores of NPMDS/NMDAS[19]. Therefore, I first investigated the association between the mtDNA mutant load at genetic diagnosis and clinical variables and then with functional scale changes at 4 different time points (symptom onset, year 1, 2nd stroke-like episode and the last visit) to reflect degenerative disease course of MELAS. This study revealed that the mutant load at genetic diagnosis was inversely correlated with age at symptom onset, age at seizure onset, and age at first clinical and MRI-confirmed stroke-like episode (all P<.0001, respectively). The mtDNA mutant load also negatively correlated with worse abnormality in MRS study and maximal serum lactic acidosis level (P=0.0032 and

P=0.0007, respectively). From this result I can infer that the higher blood mutant load early in the diagnosis reflects higher disease burden that leads to earlier onset of certain clinical manifestations, as shown by early onset initial symptoms, seizure, clinical stroke-like episode, and MRI positive stroke-like episode, and elevation of serum lactate levels, and abnormal findings in MRS. Attention should be paid to seizure, stroke-like episode, serum lactic acidosis, and imaging study related changes in patients who have high blood mutant load early in the disease course.

When it comes to the association between blood mutant load and functional scales, generally, mtDNA mutant load did not significantly correlate with any of the 6 functional scale absolute scores at any point in disease progression. Further correlation studies revealed the following results, but these observations were not consistent with disease progression. The blood mutant load correlated positively with significance with Function_total at symptom onset (r=0.5476, P=0.0046) but this trend did not persist through disease progression. This result may suggest that a higher blood mutant load early in the disease course relates to worse overall function at symptom onset, consistent with aforementioned correlation results with clinical variables. However, the blood mutant load could not be reflected following Function_total scale scores, or in other words, Function_total scale scores were within too narrow score range to reflect subsequent changes. The blood mutant load did not significantly correlate with Neuromuscular_total at symptom onset but showed significantly positive correlation with that at the last visit (r=0.4418, P=0.027). Higher blood mutant load may not relate to worse neuromuscular function early in the disease course, but as time progress, it may lead to a cumulative effect on neuromuscular manifestation with complex and repetitive damage from recurrent stroke-like episodes, epilepsy, ataxia, and myopathy. High blood mutant load at genetic diagnosis predicts a bad overall function in the beginning and consequential bad neuromuscular function at follow up. This did not apply to non-neuromuscular_total, suggesting that the sum of the involvement of other organs may not accurately reflect disease burden, because of heterogenous involvement of non-neurologic organs throughout the body with varying severities.

As no definitive trend was observed to indicate a correlation between the blood mutant load and functional scale score absolute values at each time point, I further investigated the changes in functional scales in between each time point (slope changes) and their correlation with the mutant load. As stated earlier, a significant correlation between blood mutant load and Neuromuscular_total at the last visit was found, and I also separately calculated the neuromuscular portion from the NPMDS (n=16) and NMDAS (n=9) for comparison. Not all patients suffered 2nd stroke-like episode in the current study therefore, disease progression was defined as follows: symptom onset – year 1- 2nd stroke-like episode – last visit (n=21) or symptom onset – year 1 – last visit (n=25) or symptom onset – 2nd stroke-like episode – last visit (n=21). This investigation revealed that mRS, NPMDS, NMDAS, Function_total and Non-neuromuscular total do not show a statistically significant correlation trend with disease progression. However, there was a statistically significant positive correlation between the blood mutant load at genetic diagnosis and ΔNeuromuscular_total (slope) (n=25) with disease progression, from symptom onset through year 1 until the last visit, considering the presence of 2nd stroke-like episode or not (r=0.5075, P=0.0096; r=0.532, P=0.0062; r=0.4698, P=0.0178, respectively). This trend continued also with positive correlation with statistical significance when calculated by disease duration of 1 month (r=0.4284, P=0.0326; r=0.428, P=0.0328; r=0.4012, P=0.0468, respectively). However, the same trend was not apparent with ΔNPMDS_neuromuscular (n=16) and ΔNMDAS_neuromuscular (n=9). This result suggests that the simplified and modified version of Neuromuscular_total reflected worsening neuromuscular function over time with disease progression in relation to blood mutant load at the time of genetic diagnosis, but the absolute values calculated at each point did not correlate apart from the last visit with worse neuromuscular function. Thus, these results suggest that the higher blood mutant load early in the disease course leads to a faster increase in ΔNeuromuscular_total (slope) with statistical significance and strong positive correlation, which implies that these patients may show deterioration in neuromuscular aspects at ongoing follow-ups. As previously mentioned, the neuromuscular manifestations in MELAS patients are

diverse, variable in severity and complicated by repetitive cortical damage and additional neurodegenerative process with resultant debilitation[3]. This ongoing decline in neuromuscular aspects might have bigger impact on disease burden anticipated from the mutant load early in the disease course, affecting mental and physical health and increasing care burden for both the patient and the family or caregivers. Considering this, measurement of blood mutant load early in the disease course and anticipatory evaluation of neuromuscular function may be helpful in timely appropriate intervention and counseling in advance in MELAS population.

My study has several limitations. First, this study was conducted in a retrospective fashion in some of the subjects earlier in the study period. Secondly, as earlier mentioned, I have measured mtDNA A3243G mutant load only from the blood leukocyte as a single measurement at the time of genetic diagnosis. To comprehensively evaluate the mutant load and its change over time and during the aging process, future studies involving repetitive measurements with the same subjects would be required, in relation to regular clinic visit or important time point according to the natural history of MELAS. This may well be done together with functional scale evaluation, and if available, additional mutant load analysis with less invasive urine epithelial cells may provide further insight with comparison. It was difficult to figure out the reason why conventional and validated functional scale scores of NPMDS and NMDAS did not correlate with blood mutant load whereas simplified and modified version of Neuromuscular_total correlated with statistical significance, and with time changes. Other studies using NPMDS and NMDAS have shown that these two scales can be applied on a regular basis reflecting worsening disease progression with time, but correlation with mutant load from different samplings have not been clearly studied apart from research by Grady et al. that showed that the blood leukocyte mutant load has the least variability and correlates with disease progression[19]. My study data provides a new insight into the correlation of blood mutant load and clinical disease progression focusing on the neuromuscular aspects of MELAS. Further research with a larger number of patients and not only MELAS will be necessary, in addition to the validation research on new scales

Funtion_total, Neuromuscular_total, and Non-neuromuscular total.

V. CONCLUSION

In conclusion, the blood mtDNA mutant load obtained at the time of genetic diagnosis (earlier in the disease course and close to first clinical stroke-like episode) in patients who were clinically symptomatic and had mtDNA A3243G pathogenic mutation, the higher mutant load the earlier symptom onset, seizure onset, and stroke-like episode age, reflected worse clinical severity from the beginning. In addition, even though previously validated functional scales, mRS, NPMDS, and NMDAS reflect changes with time in these populations with statistical significance, yet they do not correlate with blood mutant load. However, modified and simplified version of Neuromuscular_total reflected disease progression with significant correlation with blood mutant load, which may enable better clinical decision making and provisions for expectational counseling in these patients. The current study was limited by the lack of validity for the modified and simplified versions of new functional scales which need further validation with a larger number of mitochondrial disease patients, but not only in MELAS.

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ABSTRACT (IN KOREAN)

미토콘드리아 DNA A3243G 돌연변이 부하와 임상적 상관관계

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배경: 미토콘드리아 뇌근병증, 젖산 산증, 그리고 뇌졸중양 에피소드 (멜라스) 증후군은 미토콘드리아 DNA 변이를 통해 유전되는 가장 흔한 종류의 뇌증 중 하나이다. 멜라스 증후군은 반복적인 뇌졸중양 에피소드와 동반되는 신경계 외 장기, 즉 심장, 콩팥, 장, 내분비계의 다양한 정도의 침범으로 인해 지속적으로 퇴행성 경과를 보인다. 멜라스 환자의 80% 정도가 미토콘드리아 DNA 뉴클레오티드 3243 위치의 tRNA leucine (UUR) 유전자의 A부터 G로의 치환에 의한 특정 유전자 변이에 기인하며, 질병을 일으키는 미토콘드리아 DNA 유전자는 돌연변이 부하에 따라 다양한 표현형을 일으킨다고 알려져 있으나, 명확한 관계는 밝혀져 있지 않다. 이에 본 연구자는 차세대 염기서열 분석법 (Next generation sequencing)을 사용하여 A3243G 유전자 돌연변이 부하에 대해 정량적 분석을 진행하고, 전반적 기능적 상태, 신경근 기능적 상태, 비신경계 기능적 상태와의 연관성을 연구하고자 하였다.

방법: 본원에서 추적관찰 중인, 임상적으로 멜라스 증후군에 합당한 환자들 57명 중 32명에서 미토콘드리아 DNA A3243G 돌연변이가 확인되었다. 최종적으로 그 중 25명 환자의 돌연변이 진단 당시의 혈액 샘플로 돌연변이 부하에 대한 연구를 차세대 염기서열 분석법으로 진행하였다. 전체 대상자들의 임상 지표, 미토콘드리아 질환 진단에 관련된 혈액학적, 근육 조직 검사 및 영상학적 검사 결과를 조사하였다. 또한 기존에 알려진 세 가지 기능적 평가 척도 - modified Rankin Scale (mRS), The Newcastle Paediatric Mitochondrial Disease Ratings Scale (NPMDS) 그리고 The Newcastle Mitochondrial Disease Adult Scale (NMDAS) 및 본 연구를 위하여 자체 개발한 단순화하고 수정한 버전인 “Function_total”, “Neuromuscular_total”, “Non-neuromuscular_total”을 멜라스 증후군 질환 진행의 네 가지 시점 (증상 발현, 1년 후, 2번째 뇌졸중양 에피소드, 마지막 내원)에 적용하였고, 이 결과 및 질환 진행에 따른 변화와 미토콘드리아 DNA 돌연변이 부하와의 상관관계를 확인하였다.

결과: 유전학적 진단 당시의 혈액에서 차세대 염기서열 분석법으로 정량적 분석한 미토콘드리아 DNA 돌연변이 부하는 60.2±18.8% (22.5-100)로, 이 결과는 증상 발현 나이, 경련 시작 나이, 첫번째 임상적 또는 MRI 확진된 뇌졸중양 에피소드 나이와 모두 통계적으로 유의한 음의 상관관계를 보였다 (각 $P<.0001$). 돌연변이 부하는 MR spectroscopy에서의 비정상 영역의 합산과 최대 젖산 산증의 수준과도 유의한 음의 상관관계를 보였다 ($P=0.0032$ 그리고 $P=0.0007$). 앞서 언급된 총 여섯 가지 기능적 평가 척도는 네 가지 시점의 변화 (증상 발현, 1년 후, 2번째 뇌졸중양

에피소드, 마지막 내원)에 따라 모두 통계학적으로 유의한 양의 상관관계를 보이며 증가하였으나, 각 평가 척도의 절대값은 유전학적 진단 당시의 미토콘드리아 DNA 돌연변이 부하와 일관된 연관성을 보이지는 않았다. 다만, “Function_total”은 증상 시작시에 돌연변이 부하와 유의한 양의 상관관계를 보였고 (r=0.5476, *P*=0.0046), 이는 질병의 진행 경과 동안 일관되게 유지되지는 않았다. “Neuromuscular_total”의 경우에는 오히려 마지막 내원시의 돌연변이 부하와 유의한 양의 상관관계를 보였다 (r=0.4418, *P*=0.027). 이에 따라 추가적으로 각 기능적 평가 척도의 시점 사이의 변화량(Δ)과 돌연변이 부하와의 상관관계에 대해 연구를 진행하였다. 나머지 기능적 평가 척도에서는 변화량(Δ)이 돌연변이 부하와 유의한 상관관계를 보이지 않았다. 그러나 Δ”Neuromuscular_total” 의 경우에는 질병의 진행경과에 따라 돌연변이 부하와 통계학적으로 유의한 양의 상관관계 (증상 발현-1년 후-2번째 뇌졸중양 에피소드-마지막 내원시 r=0.5075, *P*=0.096; 증상 발현-1년 후-마지막 내원시 r=0.532, *P*=0.0062; 증상 발현-2번째 뇌졸중양 에피소드-마지막 내원시 r=0.4698, *P*=0.0178) 를 보였으며, 이 경향성은 단위 시간차를 1개월로 보았을 때도 유효하게 유지되었다.

결론: 미토콘드리아 DNA A3243G 돌연변이가 확진되고 임상적으로도 합당한 유증상의 멜라스 환자군에서, 유전학적 진단 당시의 혈액으로부터 얻어진 미토콘드리아 DNA 돌연변이 부하 (질병의 진행에 있어 초기 시점이며 첫 뇌졸중양 에피소드와도 가까운 시점)가 높으면 높을 수록 증상 발현 나이, 경련 시작 나이, 뇌졸중양 에피소드 시작 나이가 빠르며, 이는 돌연변이 부하가 높을 수록 질병 초기부터 임상적 중증도가 높음을 나타낸다. 기존에 알려진 기능적 평가 척도인 mRS, NPMDS, NMDAS 및 이번 연구에서 새로 개발한 “Function_total”, “Neuromuscular_total”,

“Non-neuromuscular_total”은 모두 멜라스 환자군에서 질병의 진행에 따라 통계학적으로 유의한 차이를 반영하나, 그 절대값이 혈액의 돌연변이 부하와의 상관관계를 보이지는 않는다. 다만, “Neuromuscular_total”이 질병의 진행에 따라 시점 사이의 변화량 (Δ)을 통해 돌연변이 부하와 양의 상관관계를 보이며, 이는 돌연변이 부하가 높을수록 각 시점 사이의 신경근 기능 변화가 크며 이는 곧 빠르게 악화되는 신경근 기능을 반영한다. 멜라스 환자군에서 연속적 신경근 기능 평가가 돌연변이 부하와 함께 고려되었을 때, 적극적이고 빠른 치료 개입과 상담이 가능할 수 있을 것으로 생각된다.

핵심되는 말: 멜라스 (MELAS); 미토콘드리아 질환; A3243G 돌연변이; 돌연변이 부하; 신경근 기능