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# Distinctive distribution of brain volume reductions in MELAS and mitochondrial DNA A3243G mutation carriers: A voxel-based morphometric study

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ABSTRACT

*Objective:* The aim of this study was to investigate the clinically latent brain atrophy of patients with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) harboring a mitochondrial DNA A3243G mutation (A3243G) and A3243G carriers without stroke-like episodes (SEs).
*Methods:* We used voxel-based morphometry (VBM) with magnetic resonance imaging to investigate gray matter (GM) and white matter (WM) volume reductions in four MELAS patients and in five A3243G carriers compared to 16 healthy controls. In addition, we investigated the regions of previous SEs using conventional MRI.
*Results:* All four MELAS patients showed significant GM volume reductions in the left superior parietal lobule (SPL), right precuneus, right middle temporal gyrus (MTG), and bilateral posterior lobes of the cerebellum. These areas of GM volume reduction were beyond the regions of previous SEs. As for A3243G carriers, GM volume reductions in the left SPL, right precuneus, right MTG, and bilateral posterior lobes of the cerebellum were detected in three, one, two, and five subjects, respectively. All four MELAS patients showed significant WM volume reductions in the bilateral or unilateral temporal sub-gyral regions, which were included in the regions of previous SEs. No A3243G carriers showed WM volume reductions.
*Conclusion:* The distribution patterns of GM volume reductions in VBM may reflect a common vulnerability of the brains among MELAS patients and A3243G carriers.


## 1. Introduction

Mitochondrial DNA (mtDNA) transfer RNA$^{Leu(UUR)}$ 3243A>G mutation (A3243G) is the most common pathogenic mtDNA point mutation and is maternally inherited (Schapira, 2006). Heteroplasmy, which is a variation in the intracellular percentage of normal and mutant mtDNA, can lead to diverse clinical phenotypes (Schapira, 2006). Approximately 80% of cases of mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) harbor the A3243G mutation (Goto et al., 1990). In MELAS, recurrent stroke-like episodes (SEs) may progressively damage the brain and cause neurological and neuropsychological deficits (Kaufmann et al., 2011). Most A3243G carriers lack SEs and may show other phenotypes including diabetes mellitus, hearing loss and cardiomyopathy (van den Ouweland et al., 1992; Casali et al., 1995). Prospective studies demonstrated that some A3243G carriers converted to MELAS, but many others had no SEs during their observation (Weiduschat et al., 2014).

Conventional magnetic resonance imaging (MRI) studies in MELAS showed not only episodic multifocal signals, typically in cortico-subcortical parieto-occipital lesions, but also progressive cerebral and cerebellar atrophy (Kim et al., 1996; Sue et al., 1998). Furthermore, several MRI studies demonstrated that A3243G carrier also showed brain atrophy (Damian et al., 1998; Lien et al., 2001; Kobayashi et al., 2005; Fromont et al., 2009). It is plausible that chronic abnormalities caused by A3243G might cause brain atrophy regardless of the presence or absence of SEs. Despite these observations, it is uncertain to what extent brain atrophy occurs in A3243G carrier or whether there is common distribution of atrophic changes between MELAS and A3243G carrier.

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Voxel-based morphometry (VBM) is an automated technique that allows the whole-brain comparisons between two groups or between an individual and a group to identify regions of significantly different activation (Ashburner, 2007). Currently, little is known about brain morphological difference between MELAS and A3243G carrier. Here, we applied VBM analysis to brain MRI images in patients with MELAS and A3243G carriers. In addition, we also investigated the regions of previous SEs in patients with MELAS using conventional MRI to assess their influences on VBM results. Our aim was to define the clinically latent brain atrophy in MELAS and A3243G carrier compared to a healthy control group.

## 2. Materials and methods

### 2.1. Participants

We retrospectively investigated patients harboring A3243G who underwent head MRI. The data were collected between April 2012 and July 2014. Based on the proposal by MELAS study group in Japan (Yatsuga et al., 2012), the diagnostic criteria for MELAS were defined as lactic acidosis in addition to at least one episode of neurological or radiological manifestations compatible with SEs. The conventional MRI data at the acute phase of SEs, including diffusion-weighted images (DWI) and fluid-attenuated inversion recovery (FLAIR) images, were collected to identify the regions of SEs. Patients harboring A3243G who did not fulfill the diagnostic criteria for MELAS were noted as A3243G carriers. In other words, A3243G carriers had no history of SEs but suffered from the extra-neurological symptoms of mitochondrial diseases, such as diabetes mellitus, hearing loss and cardiomyopathy. In addition, A3243G carriers also included asymptomatic maternal relatives of genetically confirmed patients with A3423G. In the healthy control group, inclusion was dependent on the following: (1) 15–60 years old of age; (2) no medical history of stroke, traumatic brain injury, or psychiatric disorder, and no focal deep white matter abnormalities on MRI; (3) no family history of mitochondrial diseases; (4) normal neurological and general examinations; (5) mini-mental state examination (MMSE) score of >27; (6) no dementia as defined by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV); and (7) a Clinical Dementia Rating scale (CDR) global score of 0. All neuroimaging, clinical, and laboratory assessments were conducted at the Department of Neurology, Nagoya Daini Red Cross Hospital. Subjects underwent neuropsychological assessments using the MMSE, frontal assessment battery (FAB), and clock-drawing test (CDT). In the patients harboring A3243G, the severity of mitochondrial disease was assessed by the Japanese Mitochondrial Disease Rating Scale (JMDRS), which ranges from 0 (normal) to 81 (Yatsuga et al., 2012). Serum lactic acid (LA) and pyruvic acid (PA) levels were measured by enzymatic methods. Computed tomography (CT) of the head was performed to observe intracranial calcification. Electroencephalography (EEG) was performed to measure neural oscillations. All clinical and laboratory analyses for MELAS were performed not at the acute phase of SEs but at the time when patients were clinically stable and more than three months had elapsed after any SEs. The study protocol was approved by the ethics committee of Nagoya Daini Red Cross Hospital.

### 2.2. MRI protocol

Three-dimensional (3D) T1-weighted images were acquired on a 1.5 T scanner (OVAL ECHELON, Hitachi Medical Corporation, Tokyo, Japan). In total, 120 sagittal slices were obtained using the following parameters: TR: 10.4 ms, TE: 2.3 ms, flip angle: 15°, acquisition matrix: 256 × 256, reconstruction matrix: 256 × 256, field of view (FOV): 220 × 220 mm, in-plane resolution: 0.9 × 0.9 $mm^2$, slice thickness: 1.4 mm, no gap. In patients with MELAS, MRI scans for VBM were performed at >3 months after the last SE. In addition to 3D T1-weighted images, FLAIR images used to assess the old lesions by previous SEs.

### 2.3. VBM data analysis for volumetry

The 3D T1-weighted images were analyzed using Statistical Parametric Mapping (SPM8; Wellcome Department of Imaging Neurosciences, London, UK; http://www.fil.ion.ucl.ac.uk/spm) and VBM8 (Department of Psychiatry, University of Jena, Germany) with Diffeomorphic Anatomical Registration using Exponentiated Lie Algebra (DARTEL) running on MATLAB (MathWorks, Natick, MA, USA) (Ashburner, 2007). SPM8 analysis: For MRI group comparisons between individual patients and the 16 healthy controls using VBM, a whole brain voxel-based statistical jackknife approach was conducted (Matsuda et al., 2012). The preprocessed data were analyzed using an analysis of covariance model; age, gender, and total brain volume were considered nuisance variables. The statistical threshold for significance was $p < 0.001$ (uncorrected for multiple comparisons) with an additional cluster extent threshold of 50 voxels (gray and white matter volumes). These MRI methods were described in detail in our previous study (Senda et al., 2012). In order to assess the individual abnormal quantitative extents for each patient with MELAS and A3243G carrier in VBM, a whole brain voxel-based jackknife approach was conducted [A, B]. This consists of repeating the SPMimage statistical analysis, including each patient with MELAS and A3243G carrier versus 16 controls, for the difference and repeating the analysis.

### 2.4. Gene analysis

Gene analysis was performed in an outside laboratory (SRL, Tokyo, Japan). The mtDNA was extracted from peripheral leukocytes. The absence or presence of A3243G was evaluated by PCR-restriction fragment length polymorphism (PCR-RFLP) method (Goto et al., 1990) before July 2010 and gene analysis by fluorescence correlation spectroscopy (gFCS) method (Bannai et al., 2004) on and after July 2010. The sensitive detection of gFCS method was equivalent to that of PCR-RFLP method.

In the PCR-RFLP method, PCR was first performed to amplify the region encoding A3243G. Next, the PCR products were digested with endonuclease *Apa* I, which can cleave the A3243G mutant sequence (GGGCCC) but not the wild-type sequence (GAGCCC), and subjected to Southern blot analysis. PCR products containing the 1% and 10% A3243G in mtDNA were used as positive control. The PCR products amplified from A3243G were digested into 137 base pair (bp), whereas normal control DNA products were not digested.

In the gFCS method, first PCR was performed to amplify the region encoding A3243G. Next, sequence-specific-primer-PCR (SSP-PCR) was performed using the first PCR products as templates. In SSP-PCR, wild-type allele-specific and mutation allele-specific primers differed in a single nucleotide at the 3′ end (T and C) and labeled by different fluorescence dyes (TAMRA and ATTO). If the sample DNA contained A3243G, the mutation allele-specific primer could bind to it and started to extend. The fluorescence-labeled SSP-PCR products were anlyzed by fluorescence correlation spectroscopy (FCS) at two different excitation wavelengths using an automated fluorescence cell sorter measuring device (MF 20; Olympus, Tokyo, Japan). The percentage of unamplified primers (K1) or amplified fragments (K2) relative to the total of fluorescence-labeled molecules (K1 + K2) was calculated. The K2% for mutation alleles showed heteroplasmic ratio of A3243G.

The gene analyses were performed for patients who fulfilled the diagnostic criteria of MEALS or those who manifested extra-neurological symptoms of mitochondrial diseases, but were not performed for clinically asymptomatic carriers when the presence of A3243G was confirmed in their maternal relatives.

### 2.5. Data statistics

R software, version 2.15.1, was used for statistical analyses. Data are expressed as the mean ± standard deviation (SD). Significance was set at a $p$ value <0.05 in the clinical evaluation. Sex differences were

analyzed by Fisher's exact probability test. To estimate differences in age at evaluation, neuropsychological assessments, and clinical scales between groups, Kruskal-Wallis test was performed, followed by evaluation with the Steel-Dwass post-hoc test for multiple comparisons.

## 3. Results

### 3.1. Participant demographics

Four patients with MELAS (one man and three women), five A3243G carriers (four men and one woman), and 16 controls (nine men and seven women) were included in this study (Table 1). The gene analyses confirmed the presence of A3243G in all patients with MELAS (MELAS 1–4) and four A3243G carriers (A3243G carriers 1–4). The gene analysis was not performed for only A3243G carrier 5 because she was clinically asymptomatic and had the sibling (MELAS 2) and the mother (MELAS 4) who had genetically confirmed A3243G. While one A3243G carrier was asymptomatic (A3243G carrier 5), four carriers had diabetes mellitus (A3243G carriers 1–4), three had hearing loss (A3243G carriers 1, 2, 4), and one had with cardiomyopathy (A3243G carrier 2). The age at evaluation was not significantly different among patients with MELAS (mean ± SD, 34.3 ± 15.2 years), A3243G carriers (46.2 ± 18.5 years), and controls (47.0 ± 12.5 years) ($p = 0.240$). The male to female sex ratio was also not significantly different among the groups ($p = 0.362$). Age at first SE and the numbers of SE varied greatly among the patients with MELAS. According to obtained DWI and FLAIR images, regions of previous SEs also varied (Table 1). No SEs occurred in any of the A3243G carriers or controls. In the neurological examinations, all patients with MELAS and some A3243G carriers showed muscle weakness. Cerebellar ataxia was not observed in any patients, but aphasia was noted in three patients with MELAS. The mean MMSE score of patients with MELAS was significantly lower (20.5 ± 10.1) than A3243G carriers (29.4 ± 0.9, $p = 0.046$) and controls (29.4 ± 0.9, $p = 0.007$). The mean FAB did not show significant differences among the three groups ($p = 0.136$). The mean CDT showed a significant reduction in patients with MELAS (6.8 ± 4.6, $p < 0.001$) and in A3243G carriers (8.8 ± 1.1, $p = 0.003$), compared to controls (10.0 ± 0.0). Some MELAS patients and A3243G carriers showed a short stature, hearing loss, diabetes, cardiomyopathy, or renal failure. Elevated serum LA was observed in all four (100%) of the patients with MELAS, but none of the A3243G carriers. Elevated serum PA was observed in three (75%) of the patients with MELAS and one (20%) of the A3243G carriers. The mean JMDRS score in patients with MELAS (24.8 ± 10.8) tended to be higher than that in A3243G carriers (7.4 ± 8.0) but was not significantly different between the two groups ($p = 0.122$). Three (75%) patients with MELAS and four (80%) A3243G carriers had calcification evident on brain CT. One patient with MELAS (MELAS 1) showed no alpha activity and low voltage fast (20–30 c/s) waves on EEG. Two (50%) patients with MELAS and three (60%) A3243G carriers showed slowing on EEG (Table 1).

### 3.2. MRI volumetry results

#### 3.2.1. Patients with MELAS versus controls

Volumetric values for the gray matter (GM) of all patients with MELAS were significantly reduced compared to controls, particularly in the left superior parietal lobule (SPL) (Brodmann area 7), right precuneus (Brodmann area 7), right middle temporal gyrus (MTG) (Brodmann area 21), and bilateral posterior lobes of the cerebellum (Fig. 1 and Supplemental Table 1). These areas of GM volume reduction were beyond the regions of previous SEs (Table 1). Volumetric values for the white matter (WM) of all patients with MELAS were significantly reduced compared to controls in the bilateral (MELAS 1, 2), left (MELAS 4), or right (MELAS 3) temporal sub-gyral regions ($p < 0.001$, uncorrected for multiple comparisons at the voxel level) (Fig. 2 and Supplemental Table 2). These areas of WM volume reduction were included in the regions of previous SEs (Table 1). Neither increased GM nor WM volumes were identified in patients with MELAS compared to controls.

#### 3.2.2. A3243G carriers versus controls

GM volume reductions in the left SPL, right precuneus, right MTG, and bilateral posterior lobes of the cerebellum were shown in three (A3243G carriers 1, 4, 5), one (A3243G carrier 4), two (A3243G carriers 2, 4), and five (A3243G carriers 1–5) A3243G carriers, respectively (Fig. 3 and Supplemental Table 3). The areas of GM volume reduction in A3243G carrier were similar to those in patients with MELAS, and the degree of atrophy in the carriers was smaller than the patients. The WM volumes of A3243G carriers were not significantly reduced compared to controls on either side of the temporal sub-gyral regions ($p < 0.001$, uncorrected for multiple comparisons at the voxel level)

**Table 1**
Participants' characteristics and clinical information.

| | MELAS 1 | MELAS 2 | MELAS 3 | MELAS 4 | A3243G carrier 1 | A3243G carrier 2 | A3243G carrier 3 | A3243G carrier 4 | A3243G carrier 5 | Controls ($n = 16$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Age at evaluation (years) | 25 | 18 | 50 | 44 | 61 | 59 | 50 | 46 | 15 | 47.0 ± 12.5 |
| Gender (F/M) | M | F | F | F | M | M | M | M | F | F/M = 7/9 |
| Times of SE | 2 | 12 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| Age at first SE (years) | 16 | 14 | 45 | 42 | | | | | | |
| Regions of previous SEs | Bl-O, P, T | Bl-P, T, Rt-O | Bl-T | Lt-T | | | | | | |
| Neurological symptoms | A, MW | MW | A, MW | A, MW | − | MW | − | − | − | − |
| Neuropsychological scales | | | | | | | | | | |
| MMSE [range: 0–30] | 6 | 28 | 21 | 27 | 28 | 30 | 30 | 30 | 29 | 29.4 ± 0.9 |
| FAB [range: 0–18] | 0 | 18 | 13 | 14 | 17 | 15 | 18 | 18 | 17 | 17.3 ± 1.2 |
| CDT [range: 0–10] | 0 | 9 | 8 | 10 | 8 | 8 | 8 | 10 | 10 | 10.0 ± 0.0 |
| Systemic symptoms | S, H, D | H | H, D | H, D | H, D | H, D, C, R | D | S, H, D, R | − | |
| JMDRS [range: 0–81] | 39 | 27 | 18 | 15 | 5 | 20 | 2 | 10 | 0 | 0.0 ± 0.0 |
| Blood tests | | | | | | | | | | |
| LA (mg/dl) [NR: 4.0–16.0] | 18.7 | 60.6 | 18.7 | 19.9 | 7.7 | 15.8 | 12.3 | 8.5 | 15.6 | NA |
| PA (mg/dl) [NR: 0.3–0.9] | 0.8 | 2.3 | 0.7 | 1.1 | 0.2 | 0.8 | 0.8 | 0.1 | 1.0 | NA |
| Calcification on brain CT | + | + | + | − | + | + | + | + | − | NA |
| Basic rhythm on EEG | LVF | 9 c/s, mixed with 8 c/s | 10–11 c/s | 9–10 c/s | 9–10 c/s, mixed with 8 c/s | 9 c/s, mixed with 8 c/s | 10–11 c/s | 9–10 c/s, mixed with 8 c/s | 10–11 c/s | NA |

Data are shown as mean ± standard deviation. A = aphasia; Bl = bilateral; C = cardiomyopathy; CDT = clock drawing test; CT = computed tomography; D = diabetes; EEG = electroencephalogram; F = female; FAB = frontal assessment battery; H = hearing loss; JMDRS = Japanese mitochondrial disease rating scale; LA = lactic acid; Lt = left; LVF = low voltage fast (20–30 c/s) waves (no alpha activity); M = male; MMSE = mini-mental state examination; MW = muscle weakness; NA = not accessible; NR = normal range; O = occipital lobe; P = parietal lobe; PA = pyruvic acid; R = renal failure; Rt = right; S = short stature; T = temporal lobe.

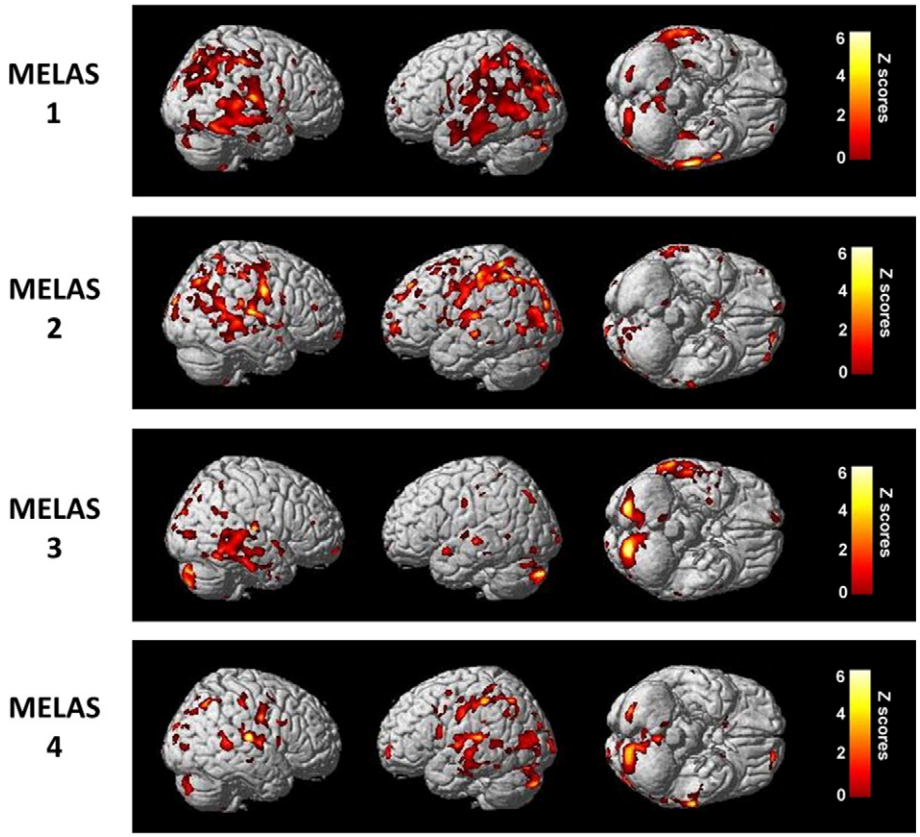


**Fig. 1.** Comparisons of individual patients with MELAS (1–4) vs. 16 controls. Gray matter volume images show the areas of volume reduction with a Z-score bar. All statistical image analyses were set at the voxel level, significant at $p < 0.001$, uncorrected for multiple comparisons. Rt: right, Lt: left.

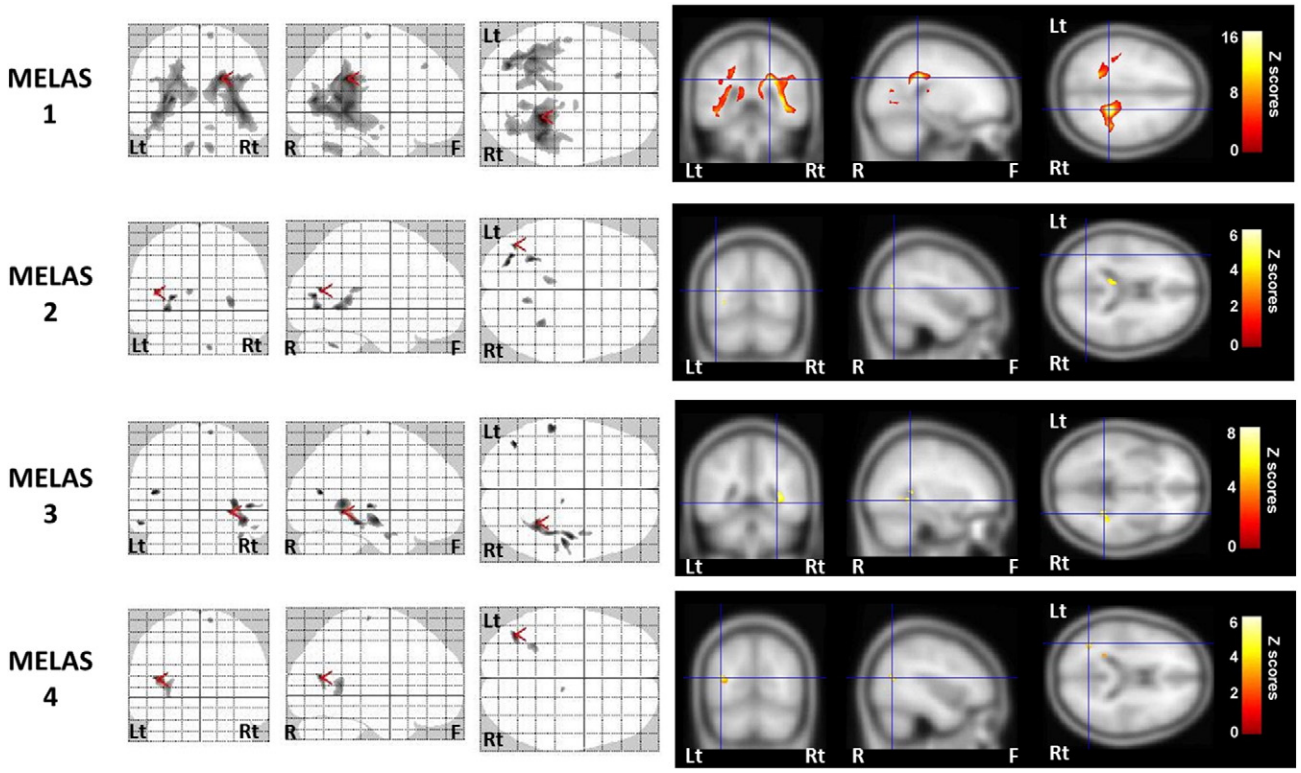


**Fig. 2.** Comparisons of individual patients with MELAS (1–4) vs. 16 controls. White matter volume images with glass brain and section depictions show the areas of volume reduction with a Z-score bar. Red arrows represent the sites of greatest reduction. All statistical image analyses were set at the voxel level, significant at $p < 0.001$, uncorrected for multiple comparisons. Rt: right, Lt: left, F: front, R: rear.

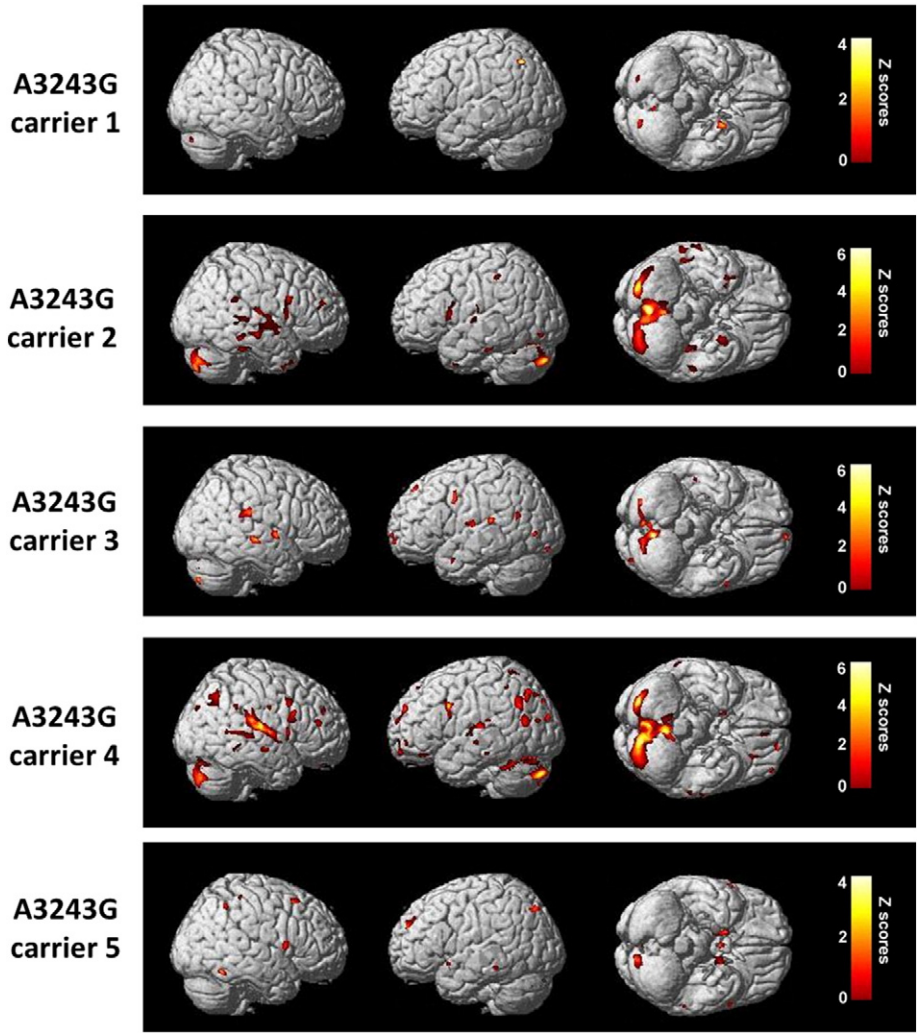


**Fig. 3.** Comparisons of individual A3243G carriers (1–5) vs. 16 controls. Gray matter volume images show the areas of volume reduction with a Z-score bar. All statistical image analyses were set at the voxel level, significant at $p < 0.001$, uncorrected for multiple comparisons. Rt: right, Lt: left.

(Fig. 4 and Supplemental Table 4). Neither increased GM nor WM volumes were identified in A3243G carriers compared to controls.

### 3.3. Gene analysis results and regions in GM volume reduction

The PCR-RFLP method was performed in MELAS 2 and A3243G carrier 1. The mutant band of MELAS 2 was stronger than positive control containing 10% A3243G, whereas the mutant band of A3243G carrier 1 was weaker than positive control containing 1% A3243G (Supplemental Fig. 1). The GM volume reductions in A3243G carrier 1 were milder than those in MELAS 2 (Supplemental Table 5).

The gFCS method was performed in MELAS 1, 3, 4 and A3243G carriers 2–4. The heteroplasmic ratios of A3243G in leukocytes was >80% in MELAS 1 (84.2%), MELAS 4 (85.8%) and A3243G carrier 4 (84.9%), but <60% in A3243G carrier 3 (59.5%). The GM volume reductions in A3243G carriers, especially in A3243G carrier 3, were milder than those in the two patients with MELAS (Supplemental Table 5). In MELAS 3 and A3243G carrier 2, the gFCS data for calculating heteroplasmic ratios was unable to be collected.

## 4. Discussion

In this study, we demonstrated that the GM volume reductions in A3243G carriers were less extensive than in the patients with MELAS, but both groups shared a common spatial distribution of GM volume reductions, including the left SPL, right precuneus, right MTG, and bilateral posterior lobes of the cerebellum. Our data indicated that there is a common vulnerability of the brains among patients with MELAS and A3243G carriers.

The sole published VBM study for patients harboring A3243G (Virtanen et al., 2011) reported the GM volume reductions mainly in the occipital pole regions, intracalcarine cortex regions and cerebellar gray matter. These data seemed to be compatible with our results. However, unlike our method, these data were obtained from the comparisons between a control group and a mixed group consisting of MELAS and A3243G carrier. Therefore, the difference of GM volume reductions in MELAS and A3243G carrier has remained to be elucidated. In this analysis, we distinguished between MELAS and A3243G carrier and revealed specific and similar distribution of the GM volume reductions in them.

In addition, we noted that the areas of WM volume reductions in MELAS were included in the region of their previous SEs, whereas the areas of GM volume reduction were beyond the regions of the SEs. For example, MELAS 4 had only one SE in her left temporal lobe and showed WM volume reductions mainly in the left temporal sub-gyral regions; however, she showed GM volume reductions beyond the left temporal lobe. The GM volume reductions in MELAS might include not only the chronic stage of SEs but also other latent abnormalities without corresponding episodic symptoms. Our data indicated that A3243G carriers could have microstructural brain damage as well as MELAS.

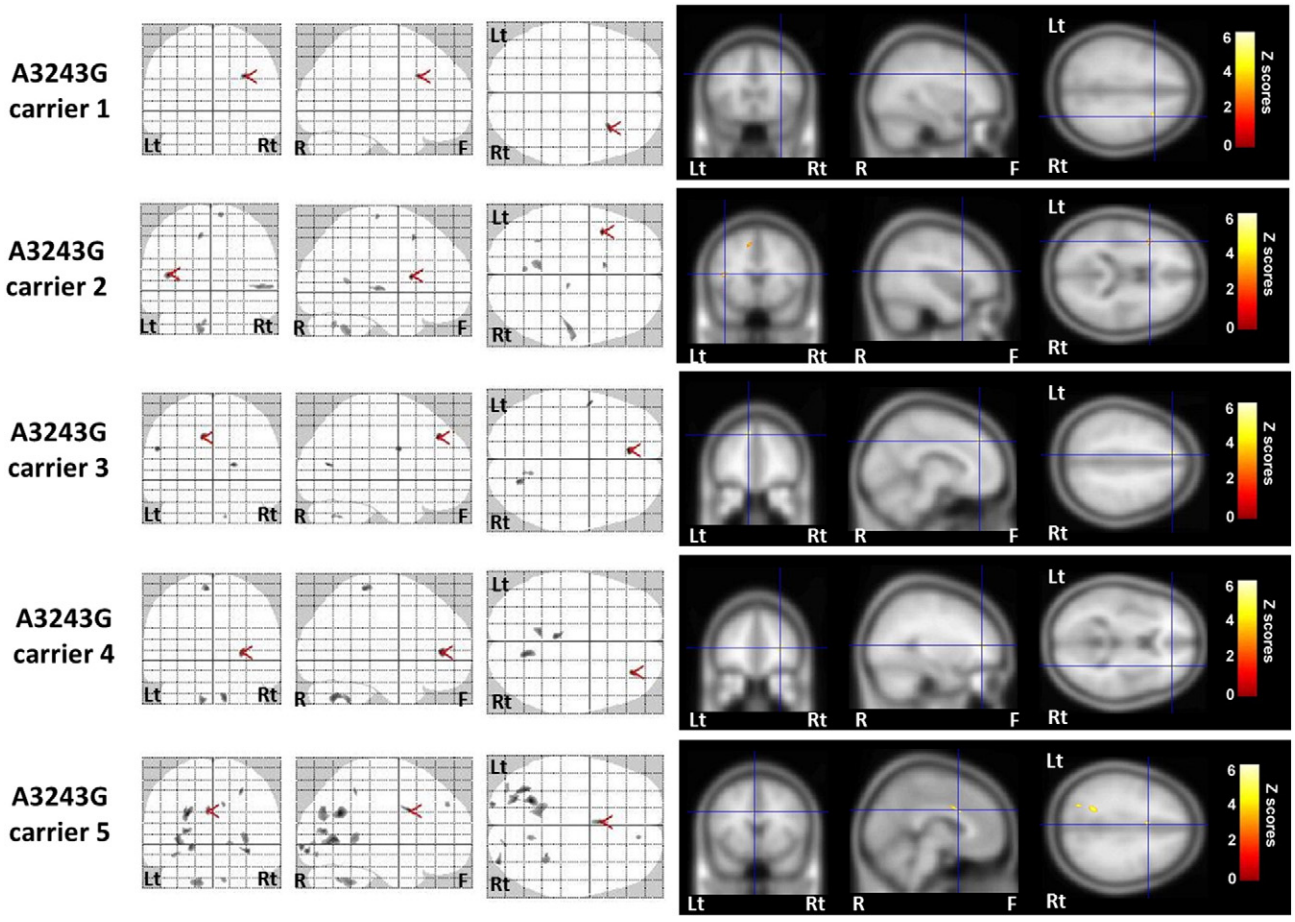


**Fig. 4.** Comparisons of individual A3243G carriers (1–5) vs. 16 controls. White matter volume images with glass brain and section depictions show the areas of volume reduction with a Z-score bar. Red arrows represent the sites of greatest reduction. All statistical image analyses were set at the voxel level, significant at $p < 0.001$, uncorrected for multiple comparisons. Rt: right, Lt: left, F: front, R: rear.

Among the atrophied regions we found, the precuneus is known to be selectively connected with other parietal areas, including the SPL, and is involved in visuo-spatial information processing (Cavanna and Trimble, 2006). The right MTG is believed to play an important functional role in the verbal labeling of emotional facial expressions (Rapcsak et al., 1993). In contrast to the relatively motor-related anterior lobes of the cerebellum, the posterior lobes of the cerebellum play an important role in cognitive behavior (Schmahmann and Sherman, 1998). The disturbances in these atrophied regions might affect neuropsychological performances in MELAS and A3243G carrier.

Previous single photon emission computed tomography and positron emission tomography studies (Grünwald et al., 1990; Damian et al., 1998; Molnár et al., 2000; Lien et al., 2001; Lindroos et al., 2009) revealed that regional hypoperfusion and glucose metabolic deficits were found with predilection for the posterior part of the brain in A3243G carriers as well as patients with MELAS. Intriguingly, we found here that the atrophic regions had some predilection for the posterior part. However, it is unclear what cause underlies the selective distribution of lesions. The pathophysiology of SEs in MELAS remains to be elucidated, but SEs are known to have predilection for the parieto-occipital areas (Kim et al., 1996; Sue et al., 1998). The posterior cerebral circulation is more susceptible to disturbances in autoregulation (Hauser et al., 1988). Thus, impaired cerebrovascular autoregulation due to abnormal mitochondria has been thought to lead the distribution of lesions (Clark et al., 1996). In addition, cortical spreading depression, which is a wave of cortical depolarization that originates in the occipital pole and is thought to produce migraine auras, was also proposed as possible mechanism causing the selective distribution of SEs (Betts et al., 2006). The mechanism similar to SEs may contribute to the selective distribution of latent atrophic regions in MELAS and A3243G carrier.

This study has several limitations. First, the number of subjects participating was rather small. To address this issue, we did not perform group-wise analysis but made comparisons between a group and an individual. This individual analysis study enabled us to observe heterogeneous volumetric changes in the patients harboring A3243G. Second, there were differences in the age and gender balance of the groups. We have included age and gender as nuisance variables and reduced any confounding effects. Third, genetic analyses were not performed for all subjects in a unified manner. Our results suggested A3243G carriers with a low heteroplasmic ratio of A3243G in their leukocytes exhibit mild brain morphological changes. The statistical analysis is needed to evaluate correlation of heteroplasmic ratio and VBM findings, but the number of available sample data was not enough for the analysis. We did not perform genetic analyses on the carriers and healthy subjects, because of ethical issues and retrospective study design. In the future, we will focus more on the correlation between structural changes, clinical and neuropsychological features, and heteroplasmic ratio with a larger number of symptomatic patients harboring A3243G, asymptomatic carriers and healthy controls matched for age and gender.

## 5. Conclusion

We used VBM with MRI and demonstrated that patients with MELAS had GM volume reductions in the left SPL, right precuneus, right MTG and bilateral posterior lobes of the cerebellum. These distributions of

GM volume reductions were beyond the regions of previous SEs and similar to those in A3243G carriers. The shared volumetric changes in MELAS and A3243G carriers might reflect a common vulnerability caused by the pathogenic A3243G mutation in the mtDNA.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mito.2016.08.011.

## Conflicts of interest

No conflicts of interest exist for any of the authors.

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