Hearing Research 428 (2023) 108678

Contents lists available at ScienceDirect

Hearing Research

journal homepage: www.elsevier.com/locate/heares

Taurine, Coenzyme Q_{10}, and Hydrogen Water Prevents Germanium Dioxide-Induced Mitochondrial Dysfunction and Associated Sensorineural Hearing Loss in mouse ☆

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ARTICLE INFO

Article history:
Received 1 April 2022
Revised 26 November 2022
Accepted 16 December 2022
Available online 17 December 2022

Keywords:
Germanium dioxide
Hearing loss
Mitochondrial dysfunction
Taurine
Coenzyme Q10
Hydrogen water

ABSTRACT

Mitochondrial dysfunction has been implicated in numerous common diseases as well as aging and plays an important role in the pathogenesis of sensorineural hearing loss (SNHL). In the current study, we showed that supplementation with germanium dioxide (GeO_2) in CBA/J mice resulted in SNHL due to the degeneration of the stria vascularis and spiral ganglion, which were associated with down-regulation of mitochondrial respiratory chain associated genes and up-regulation in apoptosis associated genes in the cochlea. Supplementation with taurine, coenzyme Q10, or hydrogen-rich water, attenuated the cochlear degeneration and associated SNHL induced by GeO_2. These results suggest that daily supplements or consumption of antioxidants, such as taurine, coenzyme Q10, and hydrogen-rich water, may be a promising intervention to slow SNHL associated with mitochondrial dysfunction.

1. Introduction

Mitochondria are the primary organ generating cellular adenosine triphosphate (ATP) and play a central role in a variety of cellular processes, including calcium signaling, reactive oxygen spices (ROS) generation, and apoptosis (Yamasoba et al., 2007). Based on these important roles, impairment of mitochondrial function has been implicated in numerous common diseases and conditions, such as cardiovascular disease, neurodegenerative diseases, metabolic disorders, and even in normal aging (Wang et al., 2016).

Mitochondrial dysfunction is typically associated with sensorineural hearing loss (SNHL). For example, in humans, several mutations and deletion in mitochondrial DNA (mtDNA) have been reported to cause both syndromic and non-syndromic forms of SNHL. Further, patients with age-related hearing loss (ARHL) have a significant load of acquired mtDNA mutations in their auditory tissues (Gopinath et al., 2009). In animal models, accumulation of mtDNA mutations by the mutator allele of the mitochondrial *Polg* DNA polymerase has shown ARHL acceleration (Kujoth et al., 2005). Even in acute SNHL, such as noise- and aminoglycoside-induced hearing loss, impairment of mitochondrial function has been shown to play an important role (Böttger and Schacht, 2013; Fujimoto and Yamasoba, 2019). Considering these findings, establishing a good animal model presenting mitochondrial dysfunction with hearing loss and discovering the methods for preventing the symptoms occurring in these animals seems to play an important role in overcoming many SNHL diseases as well as many other systemic diseases.

It has been demonstrated that chronic intake of germanium dioxide (GeO_2) both in humans and animal models causes symptoms and pathological findings similar to those in patients with mitochondrial encephalomyopathy, which is known as mtDNA mutation disease (Higuchi et al., 1989; Takeuchi et al., 1992; Asaka et al., 1995; Kim et al., 1998; Higuchi et al., 1991; Sanai et al., 1990; Wu et al., 1992; Li et al., 2001; Lin et al., 2006). For example, the skeletal muscles from rats treated with GeO_2 for 23 weeks contained numerous ragged-red fibers and cytochrome-c oxidase (COX)-deficient fibers and showed reduced enzyme activities in the mitochondrial respiratory chain, such as rotenone-sensitive NADH-cytochrome-c reductase and COX (Higuchi et al., 1991). These results suggest that GeO_2 administration can reproduce several pathological conditions caused by mitochondrial dysfunc-

☆ This article is part of the Special Issue Mitochondrial Function and Dysfunction in the Inner Ear Edited by Shinichi Someya, Anna Lysakowski, Katie Kindt, and David Raible. So, Dr. Lysakowski serve as a Guest Editor of this Special Issue.

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https://doi.org/10.1016/j.heares.2022.108678
0378-5955/

tion, which may be useful in elucidating diseases associated with mitochondrial dysfunction and their treatment.

Moreover, we have previously reported that diet supplemented with 0.5% GeO_2 caused profound SNHL associated with degeneration of the stria vascularis and supporting cells in guinea pigs (Yamasoba et al., 2006). This result indicated that GeO_2 application would be also used to create SNHL animal with mitochondrial dysfunction. Although we speculated that cochlear degeneration caused by GeO_2 intake was associated with mitochondrial damage, mitochondrial function was not investigated in this previous study.

ROS are known to be closely related to mitochondrial dysfunction. ROS are continuously produced via normal metabolism by the electron transport chain in mitochondria. In normal status, cells effectively remove ROS by their innate ROS defense systems, such as superoxide dismutase (SOD), catalase, and glutathione (Finkel and Holbrook, 2000; Raha and Robinson, 2000; Dereköy et al., 2004). However, uncontrolled leakage of ROS by irregular respiratory chain and/or decrease in the defense systems can lead to cellular dysfunction. It has been shown that the lack of SOD1 or glutathione peroxidase resulted in severe hearing loss or higher susceptibility to noise exposure, which causes excessive ROS production and induces damage to the outer and inner hair cells (Ohlemiller et al., 2000). These reports suggest that daily dietary intake of ROS scavengers may augment the defense system against ROS and thereby prevent cellular damage caused by mitochondrial damage or dysfunction.

In the current study, we investigated whether chronic intake of GeO_2 results in cochlear mitochondrial impairment and associated SNHL in CBA/J mice. Next, we investigated the effects of ROS scavengers, taurine, coenzyme Q10 (CoQ10), or hydrogen-rich water (Huxtable, 1992; Erdem et al., 2000; Qiao et al., 2015; Koh et al., 2014; Das et al., 2009; Manna et al., 2009; Alam and Hafiz, 2011; Roy and Sil, 2012; M Sikorska et al., 2014; M Sikorska et al., 2014; Sohet et al., 2009; Someya et al., 2009; Yamada et al., 2015; Ohsawa et al., 2008; Sato et al., 2008; Ohsawa et al., 2007; Hayashida et al., 2008; Yoshida et al., 2012; Fukuda et al., 2007; Nakashima-Kamimura et al., 2009; Lin et al., 2011; Fransson et al., 2021), on cochlear degeneration and SNHL induced by GeO_2.

2. Material and methods

Female CBA/J mice were purchased from CLEA Japan (Tokyo, Japan). The experimental protocol was approved by the Committee for the Use and Care of Animals at the University of Tokyo and conformed to the NIH Guidelines for the Care and Use of Laboratory Animals.

2.1. Experimental protocols

2.1.1. Experiment 1: development and analysis of mouse model of progressive hearing loss by chronic oral intake of GeO_2

Ten 2-month-old CBA/J mice were used. Five of them were given chow containing 0.15% GeO_2 for 4 months. The amount of GeO_2 was determined from a previous report using rats (Wu et al., 1992). The remaining five animals were given the normal chow serving as control. In the preliminary experiment, auditory brainstem response (ABR) thresholds were measured at 0, 2, 3 and 4 months at 2, 4, 8, and 16 kHz (Supplemental Figure 1). Animals given GeO_2 showed increase of ABR thresholds and became profoundly deaf at 4 months. Therefore, histological changes and gene expression were evaluated 4 months after the start of germanium administration. The left cochlea, muscle, and kidney was fixed with 2% PFA and 2.5% glutaraldehyde, the cochlea was additionally decalcified, and embedded in epoxy resin. Ultrathin sections were examined under transmission electron microscope. The right cochleae were used for gene transcriptional analysis of the cochlea by DNA micro array.

Another 10 two-month-old CBA/J mice were used to confirm gene expression by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Five animals were given chow containing 0.15% GeO_2 and the remaining Five animals were given the normal chow for 4 months. The cochleae were dissected and RNA was extracted.

2.1.2. Experiment 2: prevention of GeO_2 –induced cochlear damage by oral intake of ROS scavengers

Two-month-old CBA/J mice that showed auditory brainstem response thresholds within the normal laboratory range were used. Forty animals were given chow containing 0.15% GeO_2 for 3 months and assigned to one of the four groups (n = 10 each) according to the content of their drinking water: 1) water without antioxidant; 2) water containing 0.3% taurine (Wako Inc., Osaka, Japan); 3) water containing 150 μM water-soluble CoQ10 (Aqua Q10L10, Nisshin Pharma Inc., Tokyo, Japan); and 4) hydrogen water (Blue Mercury, Tokyo, Japan). The hydrogen water was placed in a closed glass vessel and changed every other day, which minimized the leakage of hydrogen from the water and maintained the concentration to be greater than 0.4 mM 1 day later (Ohsawa et al., 2007), and the remaining 10 animals were fed with normal chow and water as a control. Amounts of chow that each group ate were measured to confirm that there was no difference of the eating amounts among groups. Body weight of each animal was also measured before they were euthanized.

The 3-month ABR measurement took longer than usual to obtain the ABR threshold, and several animals died during the ABR measurement due to additional anesthesia. As a result, the final numbers analyzed were 10, 7, 9, 6, and 7 animals for the control, GeO_2±normal water, GeO_2±taurine, GeO_2±hydrogen, and GeO_2± CoQ10 groups, respectively. In this experiment, the significant protective effect of the ROS scavenger on GeO_2 was confirmed by 3 months, so we decided to euthanize the animals at 3 months instead of 4 months to avoid further discomfort to the animals, to prevent further sample loss, and to reduce the expense of the agents.

2.2. Assessment of hearing function

Detailed protocols for ABR measurements have been described elsewhere (Kinoshita et al., 2013). Briefly, two examiners who were blinded to the experiment and measured ABRs with a tone burst stimulus (2, 4, 8, 16, and 32 kHz) using an ABR recording system (Neuropack Σ MEB5504, Nihon Kohden, Tokyo, Japan). Mice were anesthetized with a mixture of xylazine hydrochloride (10 mg/kg, i.m.) and ketamine hydrochloride (40 mg/kg, i.m.). Needle electrodes were placed subcutaneously at the vertex (active electrode), beneath the left pinna (reference electrode), and beneath the right ear (ground). The sound stimulus consisted of a 15-ms tone burst, with a rise-fall time of 1 ms at frequencies of 2, 4, 8, 16, and 32 kHz. The sound intensity varied in 5-dB intervals near threshold. To obtain a waveform, 1024 tone presentations given at the rate of 17/s were averaged with the Neuropack MEB-2208 evoked potential measuring system (Nihon Kohden, Tokyo, Japan). The threshold was defined as the lowest intensity level at which a clear reproducible wave V could be observed in the trace. When an ABR waveform could not be evoked, the threshold was determined to be 110 dB SPL (5 dB greater than the maximum intensity (105 dB SPL) produced by the system). ABR thresholds were measured at 2 and 6 months of age in experiment 1 and 2 and 5 months of age in experiment 2.

2.3. Transmission electron microscopic observation of the cochlea, kidneys, and soleus muscles in animals given GeO_2

In experiment 1, animals were euthanized at the age of 6 months after the last ABR measurements. The left cochlea, muscle, and kidney were fixed with 2% paraformaldehyde and 2.5% glutaraldehyde, cochlea was additionally decalcified, and embedded in epoxy resin. Ultrathin sections were examined under transmission electron microscope.

2.4. Histological analysis of the cochlea under light microscope

In experiment 2, the cochlear pathology was examined under light microscope. Detailed preparation and examination protocols for determining cochlear pathology have been described previously (Lin et al., 2006; Kinoshita et al., 2013). Briefly, all animals were euthanized under deep anesthesia with xylazine hydrochloride and ketamine hydrochloride at the age of 5 months. The left cochlea was immersed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline overnight at 4 °C and decalcified in 10% ethylenediaminetetraacetic acid solution. The specimens were then dehydrated through a graded alcohol series and embedded in paraffin. The embedded tissues were cut into 5-μm thick sections parallel to the modiolus, and two sequential sections were mounted on glass slides and deparaffinized. Five sections at an interval of three slides (i.e., at an interval of approximately 30-μm) were stained with hematoxylin and eosin and observed under a light microscope (Nikon Eclipse E800M, Tokyo, Japan, 40× objective) to evaluate spiral ganglion cell (SGC) densities and stria vascularis degeneration in the lower-basal turn.

The number of SGCs and the area of Rosenthal's canal of the lower-basal turn were measured using Photoshop CS4 software, and SGC density (SGC number/mm^2) was calculated, as previously reported (S Someya et al., 2007). In brief, the number of SGCs in each profile were counted with computer monitors. The area of the Rosenthal's canal profile was determined in each photomicrograph by outlining the margin of bony canal using 'Select' tool. The number of the pixel of the Rosenthal's canal was measured using 'Histogram' tool. The pixels were then converted to the area by calculating the number of pixels per unit area. The density of SGC was calculated for each profile of the ganglion as the number of SGCs divided by the area of Rosenthal's canal (mm^2).

The area of the stria vascularis of the lower-basal turns was measured in digital photomicrographs using Photoshop CS4 software. The proportions of affected areas were also measured in digital photomicrographs. From these data, degeneration rate was calculated by the vacuolar degenerated area divided by the total area of stria vascularis.

2.5. Gene transcriptional analysis of the cochlea by DNA micro array

Detailed protocols for gene expression profiling analysis using Affymetrix microarray analysis have been described (Affymetrix 2004; Lee et al., 1999). Briefly, the right cochleae of the animals were used in this study. The cochleae were placed in a micro centrifuge tube, flash frozen in liquid nitrogen, and stored at −80 °C. Total RNA was extracted from the frozen cochleae by using the TRIzol reagent (Life Technologies, Grand Island, NY). We hybridized each sample to a single Affymetrix MOE 430A Gene Chip (Affymetrix, Santa Clara, CA). Signals in each image were normalized to minimize an overall variability in hybridization intensities by a global scaling method using the Affymetrix software as described in the previous report (S Someya et al., 2007). A gene was considered "expressed" if it displayed a "present" call in at least one GeneChip based on the Affymetrix "present/absent call" algorithms. All genes considered "not expressed" were eliminated from our analysis. To identify genes whose expression was significantly altered by GeO_2, each control sample ($n = 5$) was compared to each GeO_2 sample ($n = 5$), generating a total of 25 pairwise comparisons. Gene expression change was considered significant when the P value was <0.05 and the fold change was >1.2. We then used Database for Annotation, Visualization, and Integrated Discovery (DAVID) (Dennis et al., 2003) and Expression Analysis Systematic Explorer (EASE) (Hosack et al., 2003) to assign identified genes to "GO (Gene Ontology): Biological Process" categories of Gene Ontology Consortium (www.geneontology.org). We also used EASE to determine the total number of identified genes that were assigned to each Biological Process category and the total number of genes on the array in each Biological Process category and to identify "GO: Biological Process" categories statistically associated with AHL-correlated genes by performing Fisher exact tests. The Fisher exact score represents the probability that an overrepresentation of germanium-induced hearing loss-correlated genes in a certain GO: Biological Process category occurs by chance (Hosack et al., 2003). When the Fisher Exact score is < 0.05 for a given GO: Biological Process category, this gene list is considered to be specifically associated (enriched) in the Biological Process category. Gene probe sets were considered "genes" if they had been assigned a "gene symbol" annotation by DAVID.

2.6. Quantitative RT-PCR

We used the same mRNA pools for both microarray and quantitative RT-PCR analyses. Detection of mRNA was performed with an Applied Biosystems Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). Duplicate reactions for each primer set were run simultaneously in a 96-well plate using the TaqMan EZ RT-PCR kit. β-Actin was used as an internal standard. Oligonucleotide primers and MGB fluorescent probes (TaqMan Gene Expression Assays) were purchased from Applied Biosystems. Detailed protocols for analysis by qRT-PCR have been described (Someya et al., 2008). All data were reported as mean ± SEM.

2.7. Statistical analysis

Sigma Stat statistical software was used and all data were expressed as mean ± SD. ABR thresholds, HC survival rates, SGC densities, and SV thicknesses were compared among groups by one-way analysis of variance, and then pairwise comparisons were performed by using Bonferroni's test.

3. Results

3.1. Auditory and histopathological findings of animals given GeO_2

In experiment 1, CBA/J mice were orally given GeO_2-containing chows for 4 months from the age of 2 months. The ABRs examined at 2 months of age before experiments were within the normal laboratory range in all 10 animals and did not differ between animals given chows with and without GeO_2. At the age of 6 months, ABRs showed that animals given GeO_2-containing chows developed profound hearing loss at all frequencies examined, while those given normal chows maintained normal ABR thresholds (Fig. 1).

The histopathological examination showed that animals given GeO_2-containing chows exhibited marked degeneration of the stria vascularis in almost all cochlear turns, more markedly in the lower turn (Fig. 2). Animals given normal chows did not develop any of such pathologies (data not shown).

Transmission electron microscope examination revealed marked vacuolar degeneration in the stria vascularis, where almost all mitochondria contained electron-dense inclusions. Similarly, the distal tubular epithelium of the kidney and the sole muscles showed

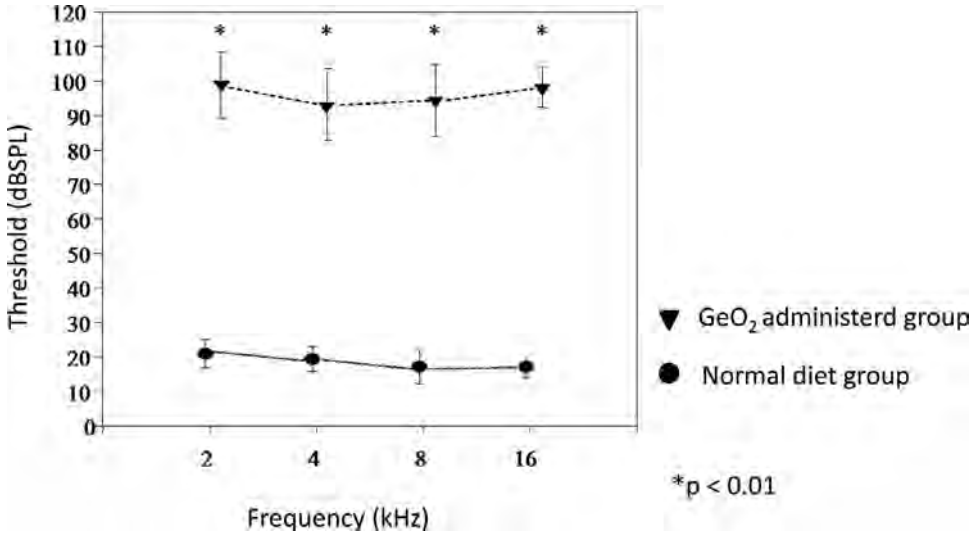

Fig. 1. Threshold after 4 months of GeO_2 treatment in CBA mice
CBA/J mice treated GeO_2-containing chows for 4 months showed profound hearing loss in all frequency (triangle), while CBA mice with normal diet showed normal hearing (circle).

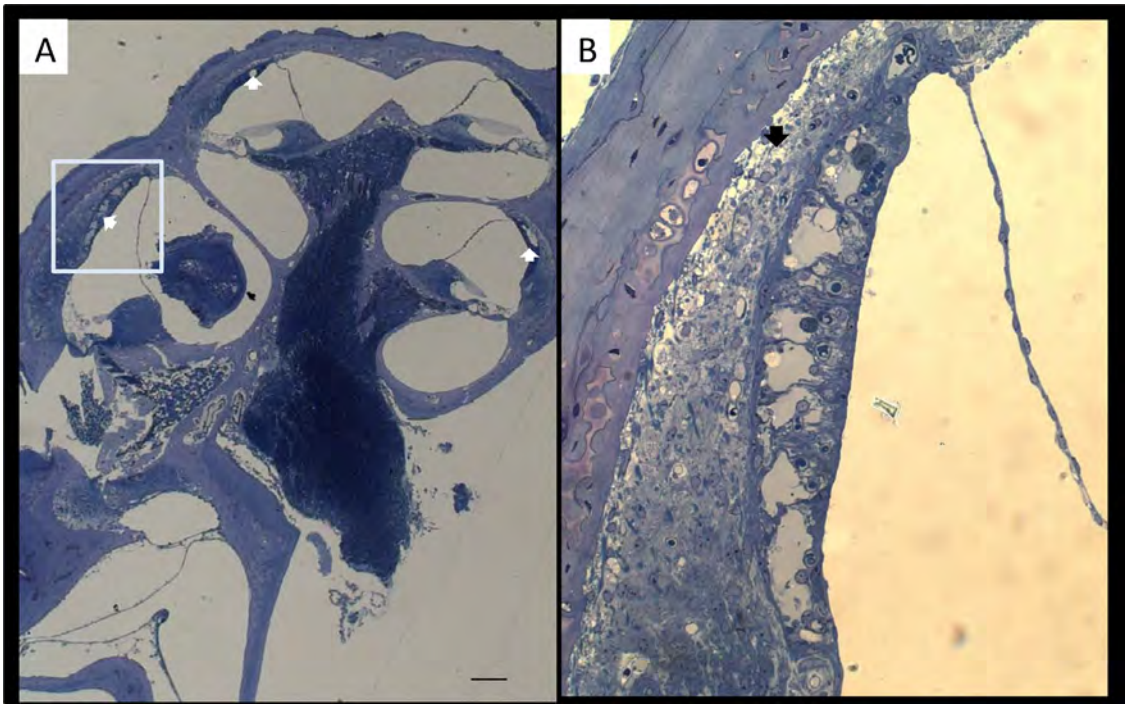

Fig. 2. Representative light micrographs of the cochlea after administration of GeO_2 for 4 months. Enlarged image of stria vascularis for captured area (B). Degeneration of the stria vascularis indicated with white arrow is seen in almost all cochlear turns, more markedly in the lower turn (A). Severe vacuolar degeneration of the stria vascularis is found in the lower basal turn (B). Bar = 100 μm in (A), 50 μm in (B).

many electron-dense deposits inside the degenerated mitochondria (Fig. 3).

3.2. Overview of microarray analysis

To identify genes and Biological Process categories associated with GeO_2-induced hearing loss, we conducted genome-wide gene expression analysis using RNA samples isolated from the cochlear tissues of 6-month-old CBA mice ($n = 5$). Using Affymetrix Gene Chip, we found that 3827 gene probe sets were significantly down-regulated, and 3327 gene probe sets were significantly up-regulated in the cochlear tissues of 6-month-old mice treated with GeO_2 compared to 6-month-old controls given normal chow. These significantly altered gene probe sets were further assigned to "GO: Biological Process" categories using Database for Annotation, Visualization, and Integrated Discovery (Huang da et al., 2009), which assigned a classification to 3827 of the downregulated and 3327 of the upregulated genes. A summary of the "Gene Ontology (GO): Biological Process" categories associated with germanium-induced hearing loss is shown in Table 1. The complete set of microarray data has been submitted to the GEO (Gene Expression Omnibus) repository (http://www.ncbi.nlm.nih.gov/geo/) with GEO Accession number GSE84735. The EASE analysis revealed that 16 Go: Biological Process categories, including "mitochondrion," "mitochondrial

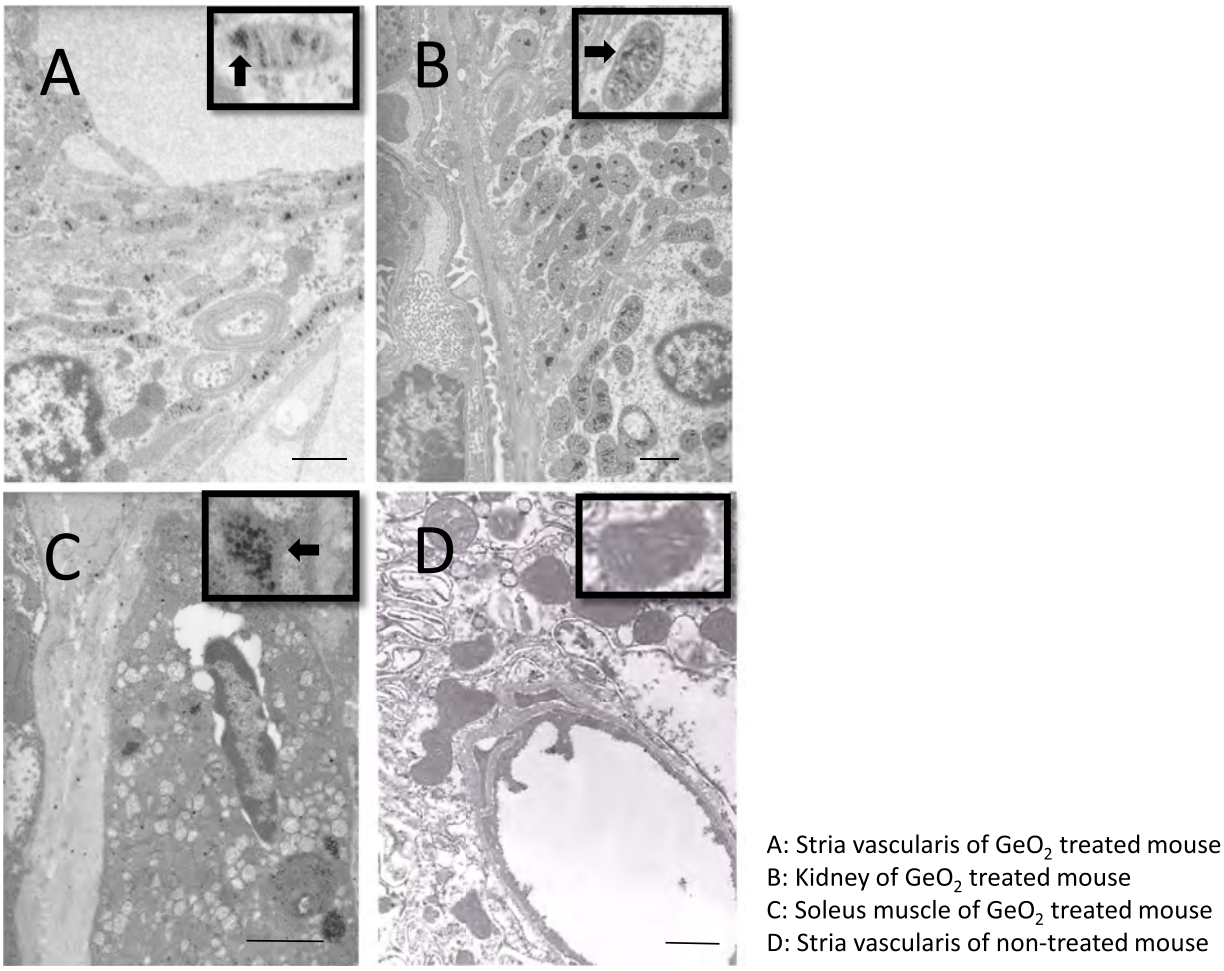

Fig. 3. Ultrastructural findings of the intermediate cells of stria vascularis (A), kidney (B), soleus muscle(C) of GeO2 treated mice. Transmission electron microscope showed vacuolar degeneration of the stria vascularis (A). The arrow head indicates degenerated mitochondria containing electron-dense inclusion. The distal tubular epithelium of the kidney (B) and soleus muscles (C) showed many electron-dense deposits inside the degenerated mitochondria. Ultrastructural findings from a stria vascularis of non-treated mice (D).Inset: high-power view of mitochondria. andBar = 1μm.

Table 1
Summary of the "GO: Biological Process" categories associated with germanium-induced hearing loss.

Biological Process Categories	N	TN	EASE
Down-regulated (3827 classified genes)			
Mitochondrion	225	679	0.000
Mitochondrial membrane	81	226	0.000
Mitochondrial envelope	85	242	0.000
Mitochondrial inner membrane	76	209	0.000
Mitochondrial electron transport chain	14	27	0.000
Mitochondrial ribosome	17	39	0.000
Mitochondrial matrix	21	57	0.001
Mitochondrial lumen	21	57	0.001
NADH dehydrogenase activity	15	35	0.001
NADH dehydrogenase (Quinone) activity	15	35	0.001
NADH dehydrogenase (Ubiquinone) activity	15	35	0.001
Tricarboxylic acid cycle	10	20	0.004
Acetyl-CoA catabolism	10	21	0.005
Oxidative phosphorylation	17	51	0.010
Acetyl-CoA metabolism	12	31	0.012
ATP binding	184	99	0.049
Up-regulated (3327 classified genes)			
Transcription, DNA-dependent	289	1418	0.000
Regulation of transcription, DNA-dependent	283	1397	0.000
Ligase activity	71	293	0.001
Endocytosis	35	1540	0.002
Apoptosis	88	433	0.018
Programmed cell death	88	439	0.025

Column titles: N, the number of identified genes in the category; FC, fold change; TN, the total number of genes in the category on the Gene Chip; EASE, EASE test score.

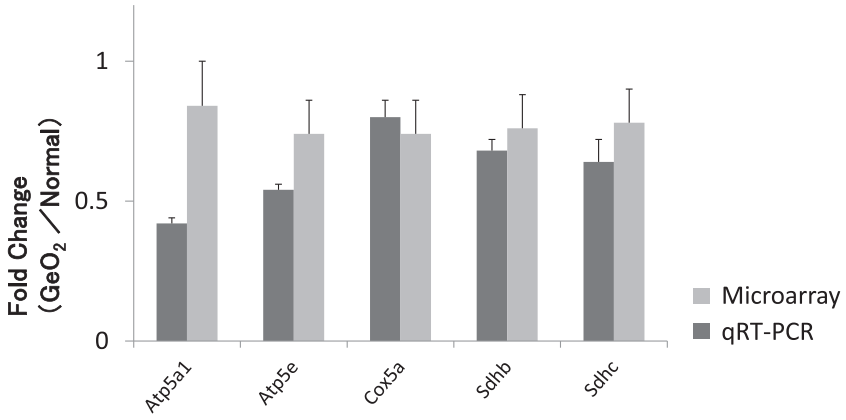

Fig. 4. The qRT-PCR validation of microarray data. The data represent the fold change in gene expression of 6-month-old mice treated with GeO_2 compared to 6-month-old controls given normal chow. The qRT-PCR analyses for *Atp5a1, Atp5e, Cox5a, Sdhb*, and *Sdhc*. The qRT-PCR results were in agreement with the microarray findings (light gray bar) that the expression of these mitochondrial function-associated genes was significantly decreased in the cochleae of germanium-treated mice (dark gray bar).

inner membrane," "mitochondrial electron transport chain," "oxidative phosphorylation," and tricarboxylic acid cycle, were significantly associated with germanium-induced mitochondrial dysfunction genes (Fisher exact score $p < 0.05$), and 818 out of 1863 genes in these categories on the Gene Chip were significantly downregulated in the cochleae of germanium-applied animals (Table 1).

3.3. Downregulation of genes associated with germanium-induced mitochondrial dysfunction

Table 2 shows a list of down-regulated genes encoding components of the mitochondrial respiratory chain in the cochlea. Twenty-eight genes encoding components of the mitochondrial respiratory chain were found to be significantly down-regulated (P value < 0.05) (Table 2). Of these, three genes encode for components of the "respiratory chain complex I" (NADH dehydrogenase complex), including *Ndufs2, Ndufs7*, and *Ndufv2*; two genes encode for components of the "respiratory chain complex II" (succinate dehydrogenase complex), including *Sdhb* and *Sdhc* genes; two genes encode for components of the "respiratory chain complex III", including *Cyc1* and *Cycs*; one gene encode for components of the "respiratory chain complex IV" (cytochrome c oxidase subunits), including *Cox5a*; and 11 genes encode for components of the "respiratory chain complex V" (ATP synthase subunits), including *Atp5k, Atp5e*, and *Atp5a1*. The analyses of qRT-PCR were conducted for *Atp5a1, Atp5e, Cox5a, Sdhb*, and *Sdhc*, to validate the microarray results. The qRT-PCR results were in good agreement with the microarray findings that expression of these mitochondrial function-associated genes were significantly decreased in the cochleae of GeO_2-treated mice (Fig. 4). These results provide the evidence that GeO_2-induced hearing loss is associated with the down-regulation of genes involved in the mitochondrial respiratory chain complexes in the cochlea of CBA/J mice.

3.4. Effect of antioxidants on ABR threshold shifts induced by GeO_2

The total amount of weekly dietary intake and final body weights for each group is shown in supplementary Table 1.

Animals given normal chow and water almost maintained ABR thresholds until 5 months of age, whereas animals given GeO_2-containing chow and normal water from 2 months of age for 3 months showed approximately 30 to 50 dB threshold shifts. The difference of the threshold shifts was significantly different between controls and animals given GeO_2+normal water at all frequencies (Fig. 5).

ROS scavengers all provided preventive effect against GeO_2-induced hearing loss, with taurine showing the strongest effect. Animal given GeO_2+taurine developed only slight threshold shifts at all frequencies. The threshold shifts in animals given GeO_2+taurine were significantly different ($p<0.01$) at all frequencies compared with those given GeO_2+normal water and were not significantly different at any frequencies compared with controls. CoQ10 prevented GeO_2-induced threshold shifts predominantly at lower frequencies, with substantial threshold shifts at higher frequencies. Threshold shifts in animals given GeO_2+CoQ10 were significantly smaller at 2, 4, and 8 kHz ($p<0.01$ in 2 kHz, $p<0.05$ in 4 and 8 kHz) compared to animals given GeO_2+normal water. Hydrogen water also provided some preventive effect, but the effect was smallest among the three ROS scavengers. The threshold shifts in animals given GeO_2 and hydrogen water showed approximately 20–30 threshold shifts at all frequencies, which were significantly different from those in animals given GeO_2+normal water only at 32 kHz ($p<0.05$). When compared among animals given GeO_2 and ROS scavengers, threshold shifts in animals given taurine were significantly smaller at 2, 4, and 32 kHz ($p<0.01$) compared to those given hydrogen water and significantly smaller at 16 and 32 kHz ($p<0.01$) compared with animals given CoQ10. Threshold shifts in animals given CoQ10 were significantly smaller only at 4 kHz ($p<0.05$) compared to animals given hydrogen water. These data indicated that taurine has the strongest preventive effect, followed by CoQ10 and then hydrogen water.

3.5. Effect of antioxidants on degeneration of the spiral ganglion and stria vascularis induced by GeO_2

The average of total area of stria vascularis at the lower basal turn for all groups are shown in Supplemental Figure 2. There were no significant differences in the average of total areas of stria vascularis among groups. Mice that did not take GeO_2 showed nearly normal appearance of the stria vascularis and SGCs at the age of 5 months (data not shown), whereas mice administered GeO_2+normal water for 3 months showed vacuolar degeneration of stria vascularis and severe degeneration of the SGCs mainly in the lower-basal and upper-basal turns in the cochlea.

The extent of degeneration in the stria vascularis and SGCs were significantly ameliorated ($p<0.01$) in animals given GeO_2 and one of the antioxidants when compared to those given GeO_2 + normal water (Figs. 6 and 7), indicative that all the antioxidants protected from GeO_2-induced cochlear degeneration. The protective effect was most significant in taurine supplementation; there was no significant difference in the extent of degeneration

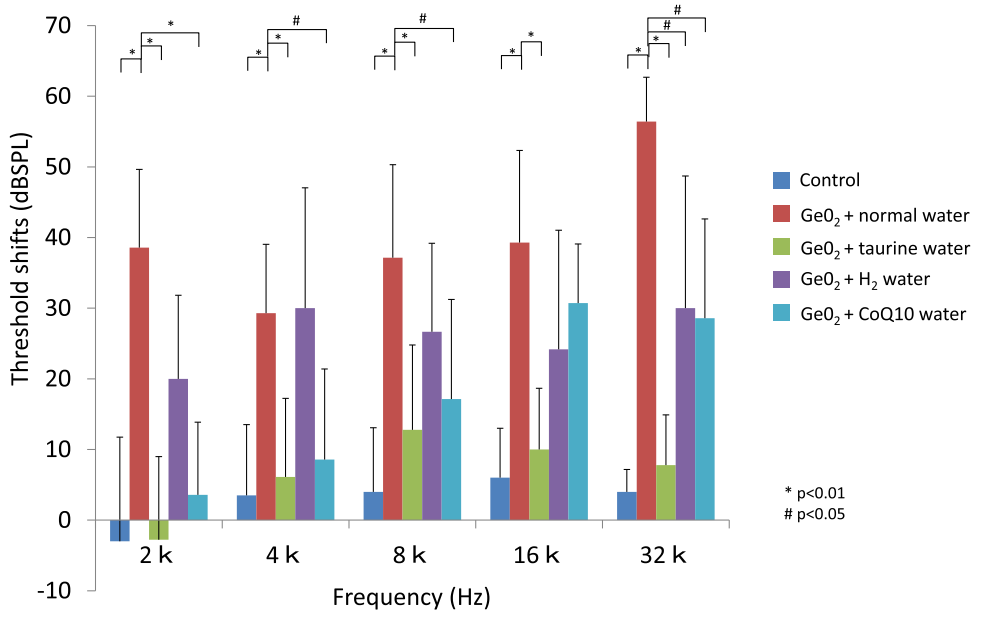

Fig. 5. ABR threshold shifts after 3 months of GeO_2 treatment
The control group showed almost no threshold shifts in all frequency. Animals given GeO_2 and water without antioxidant showed significant threshold shift in all frequency from 2 to 32 kHz. The taurine supplemented group prevented the threshold shift in all frequency in the level that was nearly the same as the control group. CoQ10 group prevented a threshold shift in most of the frequency but there was a substantial threshold shift in higher frequencies compared to control animals. Hydrogen water group had a substantial threshold shift in all frequency compared to the control group but showed some protective effect in limited frequencies compared to animals given GeO_2 +normal water.

either in the stria vascularis or SGCs between animals given GeO_2+taurine and controls without GeO_2 intake. The extent of degeneration in the stria vascularis and SGCs in animals given GeO_2+hydrogen water or CoQ10 was significantly smaller ($p<0.01$) compared to those given GeO_2+normal water, but significantly greater ($p<0.01$) compared to controls without GeO_2 intake. When compared among animals given GeO_2 and one of three antioxidants, animals given taurine showed significant protective effect against the degeneration of the stria vascularis and the SGCs when compared to those given hydrogen water or CoQ10 ($p<0.001$). There was no significant difference in the degeneration of either stria vascularis or the SGCs between animals given GeO_2+CoQ10 and those given GeO_2+hydrogen water.

4. Discussion

The current study demonstrated that oral intake of 0.15% GeO_2 for 4 months caused profound hearing loss associated with severe degeneration of the stria vascularis and SGCs in CBA mice. Transmission electron microscopic examination revealed electron-dense inclusions in degenerated mitochondria not only in the cochlea but in the kidney and muscle. Microarray gene expression analysis of the cochlea revealed down-regulation of 16 categories, including the “mitochondrion,” “mitochondrial inner membrane,” “mitochondrial electron transport chain,” and “oxidative phosphorylation.” qRT-PCR confirmed the down-regulation of five representative genes associated with mitochondrial respiratory chain in the cochlea. These findings indicate that dietary oral administration of 0.15% GeO_2 in CBA mice is a promising animal model to investigate SNHL associated with mitochondrial dysfunction. We also observed that GeO_2-induced SNHL and cochlear degeneration could be ameliorated by dietary intake of water containing taurine, CoQ10, or hydrogen, with taurine providing the strongest protection. These findings suggest that dietary intake of these antioxidants could be used to slow or treat SNHL and other phenotypes associated with mitochondrial dysfunction such as mitochondrial encephalomyopathy.

As mentioned above, we have shown that dietary intake of GeO_2 induces SNHL in CBA mice, which could be a good model to study SNHL associated with impairment of mitochondrial function. It has been reported that topical application of mitochondrial toxin, 3-nitropropionic acid (3-NP) on the round window of the cochlea can cause acute SNHL; both permanent and temporary threshold shifts were observed in this model depending on the amount of 3-NP used (Hoya et al., 2004; Okamoto et al., 2005). In the permanent threshold shift model, marked degeneration was observed in type 2 fibrocytes in the spiral prominence, type 4 fibrocytes in the spiral ligament, marginal cells, and intermediate cells in the stria vascularis 3 h after 3-NP administration, indicative that SNHL caused by topical application of 3-NP is primarily mediated by cellular degeneration in the lateral wall of the cochlea. Compared to this animal model, in our mouse model, the degeneration was observed not only in the stria vascularis but the SGCs. The difference of the affected sites may be due to the different methods of drug application. 3-NP was applied acutely and topically, whereas GeO_2 was applied chronically and systemically.

The current study revealed previously unrecognized pathways associated with GeO_2-induced SNHL, such as the down-regulation of genes involved in the mitochondrial respiratory chain. The DNA microarray analysis revealed that chronic application of GeO_2 down-regulated 27 genes in the respiratory chain complexes I, II, III, IV and V. Someya et al. (S Someya et al., 2007) reported changes of gene expression in the cochlea of DBA/2 J mice, which show severe progressive age-related hearing loss. In their study, gene analysis revealed that the aged DBA/2 J mice showed significant down-regulation of genes encoding components of the mitochondrial respiratory chain complexes I, II, III, IV, and V. Deficiency of complex IV is reported to be associated with SNHL (Horváth et al., 2005; Lamperti et al., 2012) in other reports. Gutiérrez Cortés et al. (Gutiérrez Cortés et al., 2012) also suggested that mutations of genes in complex I, III, and IV could be the cause of maternally

Table 2
List of down-regulated genes encoding components of the mitochondrial respiratory chain in the cochlea.

Gene	Gene ID	Affy ID	P Value	FC
Oxidative Phosphorylation				
Atp5a1	C78762	1,420,037_at	0.011	−2.028
Atp5d	BC008273	1,423,716_s_at	0.033	−1.571
Atp5e	NM_025983	1,416,567_s_at	0.000	−1.835
Atp5g1	NM_007506	1,416,020_a_at	0.000	−1.567
Atp5g2	NM_026468	1,415,980_at	0.002	−1.727
Atp5j	NM_016755	1,416,143_at	0.008	−1.191
Atp5k	AV216686	1,434,053_x_at	0.004	−1.727
Atp5l	NM_013795	1,448,203_at	0.009	−1.593
Atp5o	NM_138,597	1,416,278_a_at	0.000	−1.563
Atp5o /// LOC432676	AV066932	1,437,164_x_at	0.005	−1.558
Atp6ap1	AI316502	1,449,622_s_at	0.000	−1.795
Cox5a	NM_007747	1,448,153_at	0.025	−1.345
Cyc1	NM_025567	1,416,604_at	0.005	−1.266
Cycs	NM_007808	1,422,483_a_at	0.007	−1.235
Ndufc2	NM_024220	1,416,366_at	0.000	−2.120
Ndufs7	BC013503	1,451,312_at	0.001	−1.458
Ndufv2	AV046532	1,438,159_x_at	0.003	−1.259
Tricarboxylic acid cycle				
Aco1	BB504570	1,456,728_x_at	0.003	−1.360
Aco2	AU019938	1,436,934_s_at	0.047	−1.345
Cs	AB056479	1,450,667_a_at	0.022	−1.248
Dlst	BC006702	1,423,710_at	0.005	−1.616
Idh3b	NM_130,884	1,418,886_s_at	0.001	−1.316
Idh3g	NM_008323	1,416,789_at	0.000	−1.736
Mdh2	NM_008617	1,416,478_a_at	0.000	−1.342
Polr3h	AK019868	1,424,227_at	0.000	−1.181
Sdhb	BC013509	1,418,005_at	0.000	−1.387
Sdhc	NM_025321	1,448,630_a_at	0.012	−1.537

Column titles: Gene, gene symbol; Gene ID, representative public gene ID.
Affy ID, Affymetrix probe set ID; FC, fold change.

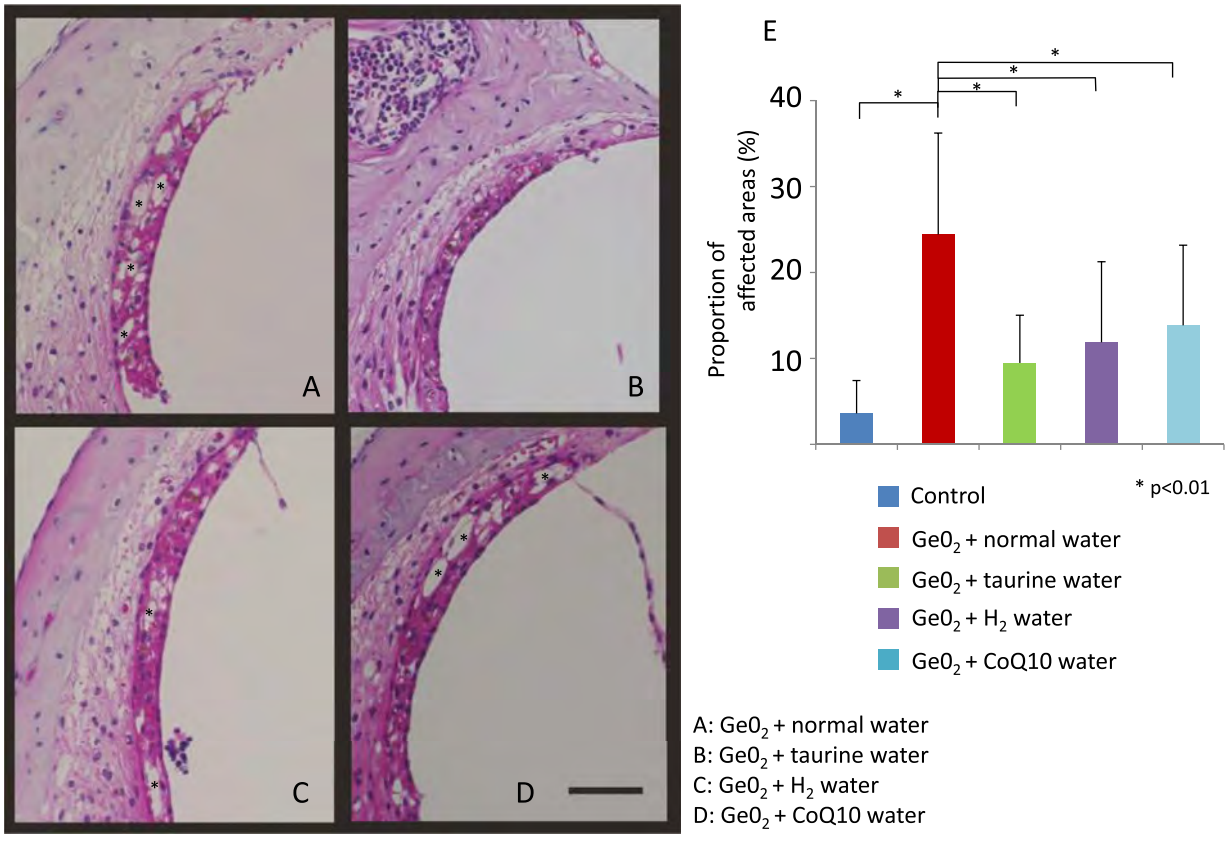

Fig. 6. Representative light micrographs of the stria vascularis in animals given germanium and water (A), taurine (B), hydrogen water (C), and CoQ10 (D). The extent of the degeneration is significantly attenuated in animals given one of the antioxidants compared to those given water without antioxidant. Taurine provides the strongest effect, with the extent of degeneration not being different compared to the controls without germanium treatment (E). Representative vascuolar degeneration area are maked with "*". Bar = 50 μm.

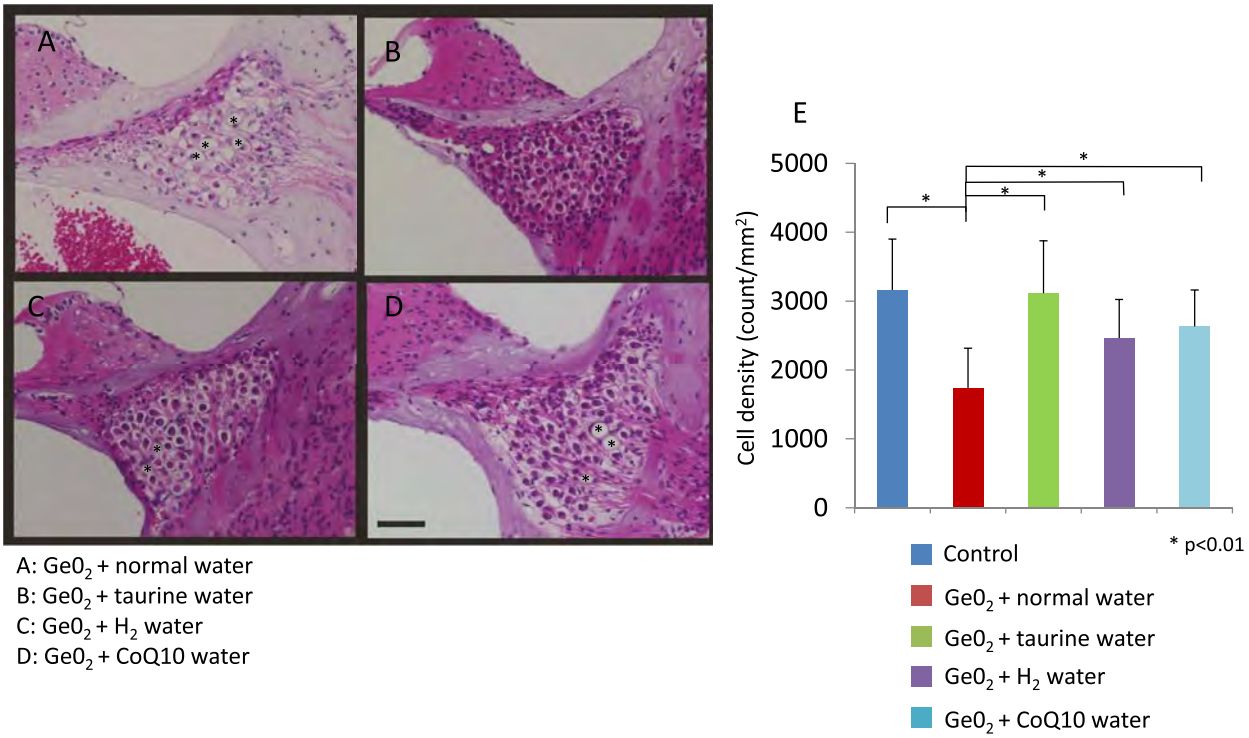

Fig. 7. Representative light micrographs of the spiral ganglion cells in animals given germanium and water (A), taurine (B), hydrogen water (C), and CoQ10 (D). The extent of the degeneration is significantly attenuated in animals given one of the antioxidants compared to those given water without an antioxidant. Taurine provides the strongest effect, with the extent of degeneration not being different compared to the controls without germanium treatment (E). Representative degenerated area are marked with "*" Bar = 50 μm.

inherited non-syndromic hearing loss. These results are in line with our findings that the dysfunction of mitochondrial respiratory chain complexes was closely related to SNHL. Moreover, the deficiencies of mitochondrial respiratory chain complexes have been reported to be closely related to neural degeneration. For example, Atp5a1 deficiency has been reported to cause severe neonatal encephalopathy, and Atp5e causes early onset lactic acidosis, 3-methylglutaconic aciduria, mild mental retardation, and severe peripheral neuropathy development. Both genes were confirmed to be down-regulated in the current study. Complex V deficiency mediated by other genes also has been observed in neurodegenerative diseases (Kantrow et al., 1997). Complex II deficiency also has been reported to cause neurodegenerative disorders (EA. Shoubridge, 2001; EA. Shoubridge, 2001). Although there has been no report examining the degeneration of SGCs, one of the peripheral neurons, in these deficiencies, it is speculated that mitochondrial dysfunction caused by the down-regulation of genes in complex I, III, and IV may be related to the degeneration of the SGCs.

In the current study, the up-regulation of genes involved in apoptosis was also observed. It has been reported that the defect of the respiratory chain system is associated with the induction of apoptosis (Kantrow et al., 1997). An in vivo study using Neuro-2A cells showed that treatment of GeO_2 to the neuron A2 cell induced the release of cytochrome c from mitochondria, loss of mitochondrial membrane potential, and translocation of the Bax, resulting in apoptosis by the mitochondrial-dependent pathway (Lin et al., 2006). Interestingly, such phenomena have also been reported by applying similar semiconductor elements, such as arsenic, indium, and gallium (Bustamante et al., 1997; Chang et al., 2003; Hu et al., 2003; Milton et al., 2004). Studies investigating the mechanism for arsenic-induced apoptosis have revealed that the apoptosis is triggered by inhibition of mitochondrial respiratory function, resulting in the induction of ROS. ROS inactivate enzymes and damage DNA molecules by the direct chemical attack on their structure (Pelicano et al., 2003; Shen et al., 2003). Considering these, it can be assumed that the accumulation of germanium in the mitochondria would affect mitochondrial respiratory function, thereby resulting in ROS generation and induced mitochondria-mediated apoptosis of the stria vascularis and SGCs.

Taken together, these reports suggest that GeO_2 accumulation causes mitochondrial dysfunction, leading to the degeneration of the cochlea and subsequent progressive hearing loss via apoptotic pathway. However, the current study does not directly demonstrate a causal relationship between mitochondrial dysfunction and cochlear degeneration, which should be evaluated by future studies.

In the current study, we observed that antioxidants, such as taurine, CoQ10, and hydrogen water, attenuated GeO_2-induced SNHL and degeneration of the stria vascularis and SGCs, which implies that ROS play a key role in GeO_2-mediated damage in the cochlea. Those antioxidants have been proven to have powerful antioxidant effects in various fields. For example, taurine has been exhibited to protect various organs from oxidative stress caused by alminium (Qiao et al., 2015), diabetes (Koh et al., 2014), and various drugs (Das et al., 2009; Manna et al., 2009; Alam and Hafiz, 2011; Roy and Sil, 2012). It has also been reported that adminisitration of taurine induces a significant reduction of intracelular ROS level and recovery of mitochondria membrane potential caused by arsenic in mouse neuroblastoma N2a cells. (Chou et al., Apr). CoQ10 has been shown to protect neuronal cells from UVB- and ROS-induced damage (M Sikorska et al., 2014), brain ischemia/reperfusion, gentamicin-induced cochlear damage and hearing loss, and hepatic oxidative stress and inflammation (M Sikorska et al., 2014). In addition, supplementation of CoQ10 has been reported to show a therapeutic effect in patients with mitrochonrial respiratory chain disorders (Hargreaves, 2014).

Hydrogen gas has shown protective effects from ischemia/reperfusion injuries in cerebral (Sato et al., 2008) and myocardial infarction (Hayashida et al., 2008; Yoshida et al., 2012), hepatic injury (Fukuda et al., 2007), cisplatin-induced nephrotoxicity (Nakashima-Kamimura et al., 2009), and noise-induced hearing loss (Lin et al., 2011; Fransson et al., 2021). Although these antioxidants showed protective effect in the current study, hydrogen showed weakest effect compared to other supplements. This may be explained by the limited concentration of hydrogen when given in water. The solubility of the hydrogen is limited and easily leaked from water. Although the glass bottle used minimized the leakage of hydrogen from water and the water was changed every other day to keep the hydrogen concentration above 0.4 mM, the dose may be not sufficient to achieve satisfactory effects. Another reason may be the unique mechanism of scavenging system of hydrogen, which selectively scavenges free hydroxyl radicals (•OH) (Sato et al., 2008; Ohsawa et al., 2007). Other types of free radicals may be more relevant to GeO_2-induced damage, and as a result, the effects of hydrogen may be limited.

5. Conclusion

Chronic dietary intake of GeO_2 in CBA mice could induce SNHL due to the degeneration of stria vascularis and the SGCs, which was associated with down-regulation of mitochondrial respiratory chain associated genes and up-regulation of apoptosis-associated genes. Antioxidant supplements, such as taurine, CoQ10, and hydrogen water, could attenuate cochlear damage and SNHL induced by GeO_2 intake. SNHL induced by oral intake of GeO_2 can be a promising animal model to investigate SNHL associated with mitochondrial dysfunction. Daily supplements of antioxidants may be one of the solutions to prevent or slow SNHL associated with mitochondrial dysfunction.

Author statement

Akinori Kashio: investigation and writing of the original draft preparation; Chikako Yamada: investigation; Kazuo Yasuhara: investigation; Teru Kamogashira: investigation; Shinichi Someya: investigation and analysis; Tatsuya Yamasoba: conceptualization, methodology, writing of the review and editing, supervision, and funding acquisition.

Acknowledgment

We thank Prof. Mitsuya Suzuki, Mr. Yoshiro Mori, Ms. Atsuko Tsuyuzaki, and Ms. Yukari Kurasawa for their technical assistance in tissue preparation including transmission electron microscopy and immunostaining.

Funding source

This study was supported by Grant Number JP20H00546, JP20K21646JP from Government of Japan Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT), and Grant Number 20FC1019 from Government of Japan Ministry of Labour and Welfare.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.heares.2022.108678.

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Sec.3 ミトコンドリア病

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1　ミトコンドリア病とは

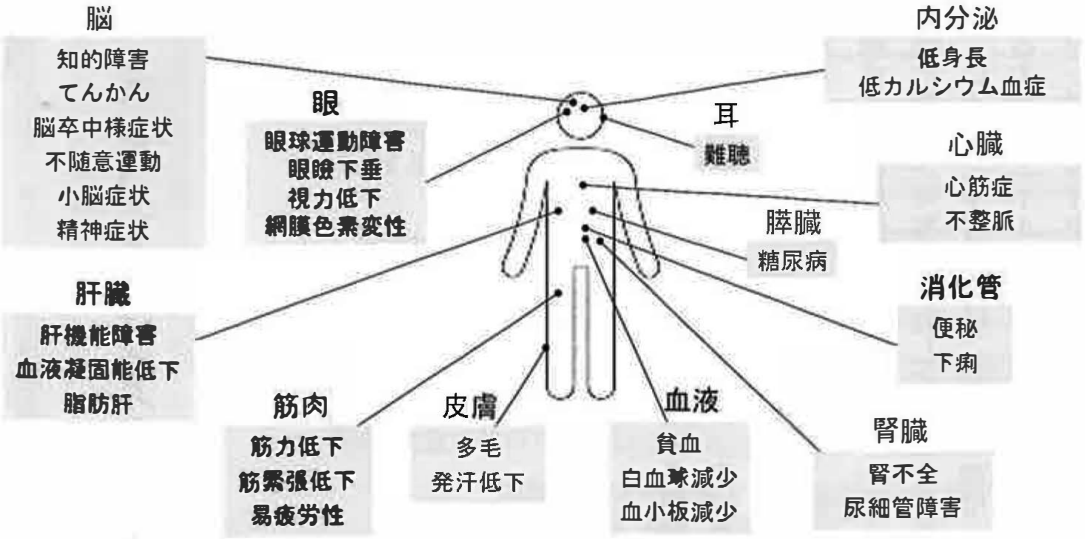
私たちの体は何10兆個もの小さな細胞が集まって形作られていますが、その細胞の一つ一つの中にミトコンドリアが存在しています。ミトコンドリアは二重膜に囲まれた細胞内小器官であり、一つの細胞に数百から数千存在していると言われています。主な役割は、細胞で利用される主要なエネルギーであるアデノシン三リン酸（ＡＴＰ）の産生です。取り込まれた栄養を利用して、細胞の、ひいてはその集合体である私たち生物の活動を支えるエネルギーを作り出す、いわば発電所のような役割を果たしています。そのため、ミトコンドリアに機能障害が生じると細胞の働きが低下して、さまざまな症状が出現します。それがミトコンドリア病です。

エネルギーを多く使う脳や筋肉などに症状が出やすいため、ミトコンドリア病は「ミトコンドリア脳筋症」と呼ばれることもあります。エネルギーを特にたくさん必要とする神経細胞の機能異常は、知的発達症（知的障害）、てんかん、不随意運動、小脳失調などの症状をもたらします。筋肉の症状としては、筋力低下、筋緊張低下、眼球運動障害、眼瞼下垂などが見られます。しかし、ミトコンドリアは体中のほぼ全ての細胞に存在しているので、ミトコンドリア病の症状は全身のあらゆる臓器・器官に現れる可能性があります。体のどの部分のミトコンドリアが障害されるかによって、個々の患者の症状は異なってきます。それぞれの臓器・器官に出やすい症状や病気を図表１に示します。

このようにさまざまな症状を呈するミトコンドリア病は、国の指定難病医療費助成制度の対象となっており、小児慢性特定疾病の一つにも定められています。指定難病として「ミトコンドリア病」と診断されるには、決められた診断基準を満たす必要があります。具体的には、筋肉、脳、心臓、肺、腎臓、膵臓、内分泌、血液、眼、耳のいずれかに、ミトコンドリア病でよく見られる症状があることが要件になります。

従来、ミトコンドリア病の患者数は非常に少ないと思われていました。しかし、近年の調査研究から、一般人口においてミトコンドリア病と診断されている患者は、以前考えられていたほど少なくないことがわかってきました。成人人口10万人あたりで、イギリスでは23人、スペインでは5・7人と報告されています。一

方、日本の正確な患者数は不明ですが、全国の入院患者の医療保険のデータベースから、平成30年度の患者数は3629人、一般人口10万人あたり2・9人と推



図表1　ミトコンドリア病の主な症状

定されています。この調査は入院患者のみを対象としているので、実際の患者数はこれより多い可能性があると思われます。

2　ミトコンドリア病の原因

ミトコンドリア病は、体の設計図である遺伝子（DNA）の変化によって引き起こされます。近年の遺伝子検査技術の進歩により、ミトコンドリア病の原因となる遺伝子の種類は極めて多いことがわかってきました。ミトコンドリアでは1500にものぼる因子（タンパクなど）が機能していると言われています。それらのうち、実に約400種類の因子において、その設計図である遺伝子の変化がミトコンドリア病患者で報告されています。

これらの遺伝子は、ほとんどが細胞の核の中に存在しますが（核遺伝子）、核の外（細胞質）にあるミトコンドリアもその内部に独自の環状DNAであるミトコンドリアDNA（ミトコンドリア遺伝子）をもっており、その変化もミトコンドリア病を引き起こします。小児期発症のミトコンドリア病では、70％～85％の患者が核遺伝子の変化、残りの患者がミトコンドリア遺伝子の変化が原因であることが知られています。一方、成人になってから発症するミトコンドリア病患者では、逆に70％～80％程度の患者がミトコンドリア遺伝子の変化、残りの患者が核遺伝子の変化が原因であると言われています。

3　ミトコンドリア病の診断

指定難病の「ミトコンドリア病」と診断されるには、先に紹介した種々の症状に加え、検査でミトコンドリアの異常を示す所見があることが基準になります（図表2）。まず、医療機関で行われる血液や髄液検査、あるいは脳画像検査や眼底検査の異常が診断基準で示されています。体内の乳酸が高くなることが多いため、血液や脳脊髄液の乳酸値の上昇は診断の一助となります。神経症状のある患者では、脳CTやMRI検査等の画像検査を行い、大脳基底核や脳幹等のエネルギー需要度の高い部位の病変の有無を調べます。眼底に特徴的所見を認めること

があるので、眼科検査も行われます。さらに、症状に応じて各臓器の評価を行い、ミトコンドリア病に特徴的な障害をきたしていないかチェックします。心臓を例にとれば、心電図や心エコーを行い、ミトコンドリア病で起こりうる不整脈や心筋症の有無を確認します。難聴や糖尿病も診断の手がかりになります。いかなる臓器・器官にも障害が起こりうることを念頭に、特徴的な所見がないか、丁寧な診察と検査によって評価することが大切です。

1．臨床検査 ①血液または髄液の乳酸値が繰り返して高い、またはMRスペクトロスコピーで病変部に明らかな乳酸ピークを認める。 ②脳CTやMRIにて、大脳基底核、脳幹に両側対称性の病変等を認める。 ③眼底検査に異常を認める（急性期の視神経乳頭の発赤・腫脹、毛細血管の蛇行、網膜神経線維腫大、視神経乳頭近傍の出血のうち１つ以上の所見を認めるか、慢性期の視神経萎縮を両眼に認める）。 2．特殊検査 ①遺伝学的検査所見：ミトコンドリアDNAに質的、量的異常、またはミトコンドリア関連分子をコードする核遺伝子変異を認める。 ②生化学検査所見：ミトコンドリア関連酵素の活性低下、またはコエンザイムQ10などの中間代謝物の欠乏を認める。またはミトコンドリアDNAの発現異常を認める。 ③病理検査所見：骨格筋生検や培養細胞または症状のある臓器の細胞や組織で、ミトコンドリアの病理異常を認める。

図表２　ミトコンドリア病の検査所見（指定難病医療費制度　診断基準を参考に作成）

　臨床的にミトコンドリア病を疑った場合には、遺伝子検査や生化学的検査などの特殊検査によって、ミトコンドリアの異常を証明します。ミトコンドリア内に存在する、エネルギーを産生するために必要な酵素の働きを評価するためには、酵素活性測定などの生化学的検査が行われます。また、症状のある臓器（筋肉や肝臓、心臓等）の組織を用いた病理学的検査によってミトコンドリア病に特徴的な所見が見られれば、診断の一助となります。そして、前述のように遺伝子の変化がミトコンドリア病の原因となるので、確定診断のためには遺伝学的検査（遺伝子検査）が大切です。原因となりうる遺伝子の数が非常に多いため、検査には時間と労力がかかりますが、近年の検査技術の長足の進歩によって、多くの患者の遺伝子診断が可能となりました。遺伝子の変化は全ての患者で検出されるわけではありませんが、ミトコンドリア病疑いの患者の60％以上で、遺伝子検査による確定診断が可能になったと言われています。

4　ミトコンドリア病の病型

　ミトコンドリア病の患者の症状は多彩です。症状によってさまざまな病気に分類されます。主な病型を紹介します。

①新生児ミトコンドリア病

　ミトコンドリア病はあらゆる年齢で発症する可能性がありますが、最も早期の新生児期、すなわち出生から1カ月以内に発症するミトコンドリア病を指します。出生直後から全身状態が悪化する重症例も多く見られます。原因としては、ミトコンドリア遺伝子よりも、核遺伝子の変化が多いことが知られています。

②Ｌｅｉｇｈ脳症（リー脳症）

　乳幼児期、多くは2歳までに発症する進行性の疾患で、脳幹及び（あるいは）大脳基底核の左右対称性の病変が特徴で

す。精神運動発達遅滞やてんかん、不随意運動、眼球運動障害や呼吸障害などの脳幹の症状が見られます。筋緊張や筋力低下といった筋肉の症状も見られます。感染症を契機にして、急激に状態が悪化することのある疾患です。患者の20％程度にミトコンドリア遺伝子異常、残りの約80％は核遺伝子異常が原因とされ、現在まで約１００種類に及ぶ原因遺伝子が報告されています。

③脳卒中様症状を伴うミトコンドリア病（ＭＥＬＡＳ／メラス）

あらゆる年齢で発症しうる、ミトコンドリア病のなかで最も頻度の高い疾患です。けいれんや意識障害、麻痺などを呈する脳卒中のような発作を特徴としますが、低身長、難聴、心筋症、糖尿病、腎症等多彩な症状を呈しうる症候群です。ミトコンドリア遺伝子の配列の３２４３番目の変化（m.3243A>G）を有する患者が80％を占めます。

④慢性進行性外眼筋麻痺症候群（ＣＰＥＯ／シーピーイーオー）

眼球運動が麻痺する、瞼が下がるなどの眼の症状が次第に進行する病気です。ＭＥＬＡＳ同様発症年齢は小児期から成人期まで幅広く、筋力低下や糖尿病、難聴など多臓器の障害を伴うことがあります。特に心臓の伝導障害（不整脈）と眼の網膜変性を合併する場合はKearns Sayre 症候群（カーンズ・セイヤー症候群）と呼ばれます。遺伝子検査では、多くの場合ミトコンドリア遺伝子の欠失（配列の一部が失われる状態）が見られます。欠失の原因が核遺伝子の変化にあることもあり、原因は多彩です。

⑤ミオクローヌスを伴うミトコンドリア病（ＭＥＲＲＦ／マーフ）

自分の意志とは無関係に筋肉が素早く収縮するミオクローヌスが最初に見られ、続いてんかん発作や体がふらつく小脳症状が出現します。筋力低下、知能低下、低身長も合併しやすい疾患です。約80％の患者に、ミトコンドリア遺伝子の配列の８３４４番目の変化（m.8344A>G）が見られます。

5　ミトコンドリア病の治療

これら多様な病型のあるミトコンドリア病に対する治療法は、大きく分けて二つあります。

一つは対症療法です。個々の患者で病型や臓器障害の種類や程度が異なりますので、それぞれの症状に応じた治療を行います。例えば、てんかんに対しては薬物によるコントロールを目指します。最近は新しいてんかん治療薬が増えているので、選択肢が広がっています。ミトコンドリア機能に悪影響を与えにくい薬剤を、発作症状にあわせて使用します。糖尿病に対するインスリン療法、難聴に対する補聴器や人工内耳、不整脈に対するペースメーカーなど、障害された臓器に対して適切な治療法が用いられます。薬物や医療機器の進歩によって、さまざまな治療法が可能となっているので、各臓器の症状、病気に応じて、それぞれの分野の専門医の診療を受けることが望ましいと考えます。そのため、多くの診療科をもつ病院を中心に医療が受けられる体制づくりが求められています。

もう一つの治療法は、ミトコンドリア病の原因であるミトコンドリア機能の低下を回復させる原因治療法です。ミトコンドリアに存在する酵素の働きを補助するビタミン類などが使用されていますが、ミトコンドリア病全般に対する十分

な効果は明らかにされていません。一方で、ミトコンドリア病が疑われる患者の一部には、ビタミン等が特異的に効果を発揮する疾患があるので、遺伝子診断などを用いた正確な診断が重要になります。

前述したMELASについては、アミノ酸の一種であるタウリンの内服治療が、脳卒中様発作の抑制効果をもつことが認められ、保険適応をもつ薬剤として承認されています。現在、脳卒中様発作以外の糖尿病などの症状に対する効果についても検討されており、今後の適応拡大が期待されます。

6　今後の課題と展望

ミトコンドリア病に対する根本的治療法はまだ十分に確立されていませんが、細胞のエネルギー産生能を高める新しい物質、抗酸化作用（異常なミトコンドリアが発する活性酸素による細胞障害を抑制する効果）をもつ薬物、ミトコンドリア遺伝子異常を減らす化合物等、さまざまな治療法開発研究が進行中です。一部の薬剤は国内外で臨床試験が始まっています。特定の病型や症状をターゲットとした治療法開発も進んでくると思われ、個々の患者に合わせた診療を実現するためには、正確な診断が一層重要になると思われます。

診断においては、遺伝子診断が重要だと述べましたが、病気の原因となる遺伝子が極めて多いことが課題となっています。多数の遺伝子を一度に調べることは技術的には可能となりましたが、それに必要な費用と労力の問題があります。また、検査で遺伝子の変化が検出されなくても、ミトコンドリア病は否定できないことにも注意が必要です。遺伝子解析技術は非常に進歩していますが、遺伝子の変化の検出に至らず「ミトコンドリア病疑い」のまま診断が定まらない患者が多いことも問題です。最近、ミトコンドリア病の遺伝子検査の保険適応が認められたので、今後、検査体制の整備が進むことで、病気の原因がわかって正確な診断に至る患者が増えることが期待されます。

まだ課題の多い状況ではありますが、診断技術が進歩して以前より多くの患者の確定診断がえられるようになったこと、新規の治療法開発の研究が進んでいることから、ミトコンドリア病診療は新しい時代に入っていると言えます。新しい治療法の効果を検証するためには、多くの患者に対する臨床試験が必要になります。また、多種多様なミトコンドリア病について、それぞれの疾患の臨床経過、患者の状況を知り、比較検討して治療効果を判断する必要があります。ミトコンドリア病は、患者数の多くない、いわゆる稀少疾患であるので、複数の医療機関・施設が協力して取り組まなければなりません。そこで、治療法開発の促進と、円滑な臨床試験実施を目的に、全国の患者を対象とした患者登録制度が運用されています。現在、小児期発症のミトコンドリア病を対象としたJ-MO Bank（http://mo-bank.com/index.html）、MELAS、CPEO、MERRF等を対象としたRemudy（http://www.remudy.jp/mitd/index.html）が利用できます。登録を希望される方は、主治医に相談していただければと思います。登録いただいた情報から、症状や経過、治療の効果を分析することで、最適な医療につながることが期待されます。