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Estimation of the Number of Patients With Mitochondrial Diseases: A Descriptive Study Using a Nationwide Database in Japan

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ABSTRACT

Background: To provide a better healthcare system for patients with mitochondrial diseases, it is important to understand the basic epidemiology of these conditions, including the number of patients affected. However, little information about them has appeared in Japan to date.

Methods: To gather data of patients with mitochondrial diseases, we estimated the number of patients with mitochondrial diseases from April 2018 through March 2019 using a national Japanese health care claims database, the National Database (NDB). Further, we calculated the prevalence of patients, and sex ratio, age class, and geographical distribution.

Results: From April 2018 through March 2019, the number of patients with mitochondrial diseases was 3,629, and the prevalence was 2.9 (95% confidence interval [CI], 2.8–3.0) per 100,000 general population. The ratio of females and males was 53 to 47, and the most frequent age class was 40–49 years old. Tokyo had the greatest number of patients with mitochondrial diseases, at 477, whereas Yamanashi had the fewest, at 13. Kagoshima had the highest prevalence of patients with mitochondrial diseases, 8.4 (95% CI, 7.1–10.0) per 100,000 population, whereas Yamanashi had the lowest, 1.6 (95% CI, 0.8–2.7).

Conclusion: The number of patients with mitochondrial diseases estimated by this study, 3,269, was more than double that indicated by the Japanese government. This result may imply that about half of all patients are overlooked for reasons such as low severity of illness, suggesting that the Japanese healthcare system needs to provide additional support for these patients.

Key words: insurance claim review; Japan; medical records; mitochondrial diseases; prevalence

INTRODUCTION

Mitochondrial diseases are caused by nuclear or mitochondrial DNA mutations, and patients vary in age of onset, sex, race, affected organ, severity, and prognosis.[1–3] While mitochondrial diseases were long thought to be rare, recent reports[4–19] have suggested that the prevalence of patients in the general population is higher than originally thought. For example, prevalence is 23 per 100,000 general adult population in the northeast of England[6] and 5.7 per 100,000 general adult population in Spain.[15] While there are few reports of mitochondrial diseases in Japan,[17,18] a questionnaire study reported a prevalence of 0.18 per 100,000 general population.[18] However, this study was limited to mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), which comprise only a proportion of mitochondrial diseases. Thus, the prevalence of overall mitochondrial diseases in Japan remains unclear.

In Japan, mitochondrial disease was recognized as an intractable disease under the designation of the Japanese Ministry of Health, Labor, and Welfare (MHLW) in 2009, which brought about various improvements in the treatment of these conditions. However, several studies have identified issues related to the treatment of mitochondrial diseases.[20,21] First, diagnosis requires a high degree of expertise. Definitive diagnosis requires an integrated approach comprising imaging, pathology, electrophysiology, genetics, and biochemical examinations, in addition to the main clinical manifestation, and the number of well-trained clinicians and hospitals equipped to perform these tests currently appears insufficient for patient needs. Second, recent developments in treatment have increased the survival of patients; in particular, the establishment of a system for transitional care from childhood to adulthood is suggested to be a major factor.[21] Third, the prevalence of some DNA mutations that cause mitochondrial diseases differs by geography.[19] This can make it difficult for patients to gain equal access to medical care, albeit that no reports of geography-related problems have appeared in Japan to date.

Accordingly, to ensure that policy makers make informed decisions and patients and their caregivers receive the best possible care, it is essential to gather basic epidemiological information on mitochondrial diseases, including the number of

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patients and their distribution by age, sex, and geographic location.

Here, we used a nationwide Japanese health claims database to estimate the number and other epidemiological parameters of patients with mitochondrial diseases.

METHODS

Data source and patient selection

We used the National Database (NDB), run by the Japanese MHLW,[22,23] to extract data on patients with mitochondrial diseases. The NDB contains information on almost all healthcare claims made from April 2009 in Japan, including patient sex; age class; numerical diagnosis code,[24] which is compatible with the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10); length of stay; costs; procedures; and prefecture where the hospital is located, among others. Data for both in- and outpatients were extracted from April 2009 through March 2019.

The MHLW permitted our use of the NDB. The study was approved by the Ethics Committee of Medical Research, University of Occupational and Environmental Health, Japan (approval number: H30-124).

Definition of mitochondrial diseases

The following diagnoses were defined as mitochondrial diseases: Pearson syndrome (compatible ICD-10 code: D640); pyruvate dehydrogenase complex (PDHC) deficiency (E744); mitochondrial disorders (E888); MELAS (E888); myoclonus epilepsy associated with ragged-red fibers (MERRF) (E888); mitochondrial neurogastrointestinal encephalopathy (MNGIE) (E888); mitochondrial cardiomyopathy (E888); mitochondrial hepatopathy (E888); mitochondrial diabetes (E888); Leigh syndrome (LS) (G318); Alpers' syndrome (G318); mitochondrial encephalomyopathy (G713); mitochondrial myopathy (G713); Leber's hereditary optic neuropathy (LHON) (H472); chronic progressive external ophthalmoplegia (CPEO) (H494); and Kearns-Sayre syndrome (KSS) (H498).

These definitions were determined by a well-trained physician and a researcher specializing in mitochondrial diseases. In sampling for this study, patients who had primary or other diagnostic positions were included, while suspected cases were excluded.

Estimation of the number of patients

The primary outcome of this study was the number of patients with mitochondrial diseases from April 2018 through March 2019. We identified affected individuals using one of the unique identifiers generated by the MHLW and assigned to individuals in NDB. This identifier consists of the health insurance number, date of birth, and sex.

From this data, we also estimated the prevalence of mitochondrial diseases. As the severity of mitochondrial diseases varies widely among patients, and some patients undergo outpatient examination only, we limited our analysis to patients who experienced at least one episode of inpatient care as representative of standard cases requiring clinical intervention above a certain level.

We calculated the prevalence of mitochondrial diseases in Japan by dividing the number of patients with mitochondrial diseases from April 2018 through March 2019 by the total population of Japan as of October 1, 2018[25] as follows:

$$prevalence\ (per\ 100{,}000\ general\ population) = \frac{(number\ of\ patients\ from\ April\ 2018\ to\ March\ 2019) \times 100{,}000}{general\ population\ on\ October\ 1,\ 2018}$$

We calculated the prevalence of mitochondrial diseases in each prefecture in a similar manner. Further, 95% confidence intervals (CIs) of prevalence were calculated using the Wald method. We also used the Wald method to calculate the 95% CI of the ratio of female patients in Japan.

Additionally, we estimated the standardized prevalence ratio (SPR) of patients in each prefecture using indirect standardization. SPR is defined as follows:

$$SPRi = \frac{Oi}{Ei} \times 100$$

Oi: *Observed number of patients in i prefecture*,

Ei: *Expected number of patients in i prefecture*

$$= \sum \left\{ \begin{array}{l} (prevalence\ of\ patients\ in\ Japan\ by\ age\ class) \\ \times\ (population\ by\ age\ class\ in\ i\ prefecture) \end{array} \right\}$$

The SPR of i prefecture is obtained by dividing Oi, the observed number of patients, by Ei, the expected number of patients. To calculate Ei, we used the prevalence in Japan as a reference population, and adjusted for age categorized into three age classes (0–14, 15–64, or ≥65 years old). The 95% CIs of SPRs were estimated using Fisher's exact CI. For example, a prefecture with an SPR of 100 has the same prevalence as Japan overall, while one with an SPR smaller than 100 has a smaller prevalence than Japan, and vice versa.

We also calculated the empirical Bayes estimator of standardized prevalence ratio (EBSPR) of each prefecture. EBSPR is defined as follows:

$$EBSPRi = \frac{Oi + \beta}{Ei + \alpha} \times 100$$

α, β: *estimator*

We estimated the EBSPR using a Poisson-Gamma model.[26] We expect that use of EBSPR should smooth out the influence of different population sizes in each prefecture on SPR.

Data analyses were performed using Stata 16.0 (StataCorp, College Station, TX, USA) and EB estimator for Poisson-Gamma model Version 2.1.[27]

RESULTS

Number of patients and prevalence of mitochondrial diseases

Within the study period, there were fewer male patients with mitochondrial diseases than female patients (47 vs 53; 95% CI for female ratio, 0.51–0.54), and the majority of patients fell within the age class 0–9 years old (Table 1). A total of 3,629 patients were diagnosed with mitochondrial diseases from April 2018 through March 2019, at a prevalence of 2.9 (95% CI, 2.8–3.0) per 100,000 general population.

Table 1. Patients' background ($n = 3,629$)[a]

	n	%
Sex, male, *n* (%)	1,712	47
Age class, years, *n* (%)		
0–4	315	9
5–9	333	9
10–14	233	6
15–19	244	7
20–29	352	10
30–39	432	12
40–49	496	14
50–59	431	12
60–69	377	10
70–79	302	8
80 over	114	3

[a]This table shows the number of patients with mitochondrial diseases from April 2018 to March 2019 identified in this study. Median age class was 30–39 years (interquartile range: 15–19, 50–59 years).

Table 2 lists the diagnosis codes and the corresponding number of patients. The majority of patients had diagnosis codes for mitochondrial encephalomyopathy and mitochondrial disorders (1,786 and 1,370, respectively).

We also compared the number of patients identified as having mitochondrial diseases in this study with the number using the Japanese medical expense subsidy system, as reported by the government.[28] The number of patients identified in this study was more than two times greater than the number using the medical expense subsidy system (3,629 vs 1,504).

Number of patients and prevalence of mitochondrial diseases in each prefecture

Table 3 and Figure 1 show the number of patients and prevalence of mitochondrial diseases in each prefecture from April 2018 through March 2019 in Japan. The prefecture with the greatest number of patients with mitochondrial diseases was Tokyo ($n = 477/3,629$, approx. 13%) while Yamanashi had the fewest ($n = 13/3,629$, approx. 1%). The prevalence of mitochondrial diseases was highest in Kagoshima (8.4/100,000) and lowest in Yamanashi (1.6/100,000).

We also compared the number of patients identified as having mitochondrial diseases in each prefecture in this study with the number using the Japanese medical expense subsidy system, as reported by the government.[28] The number of patients identified in this study was greater than the number using the medical expense subsidy system in all prefectures in Japan.

SPR and EBSPR of patients with mitochondrial diseases in each prefecture

Table 4 shows the SPRs and EBSPRs of patients with mitochondrial diseases in each prefecture. Similar to the prevalence shown in Table 3, Kagoshima and Okinawa had the highest SPRs of all prefectures, at 294 and 152.3, respectively. In contrast, Yamanashi and Saitama had the lowest SPRs of all

Table 2. Number of patients with mitochondrial diseases with each diagnosis[a]

ICD-10	Diagnosis code	Diagnosis	Total		Female	
			n	%	*n*	%
D640	8846217	Pearson syndrome	—	—	—	—
E744	8848412	PDHC deficiency	81	2	56	69
E888	8845613	Mitochondrial disorders	1,370	38	750	55
E888	8846079	MELAS	284	8	159	56
E888	8846080	MERRF	15	1	—	—
E888	8846084	MNGIE	—	—	—	—
E888	8846224	Mitochondrial cardiomyopathy	174	5	86	49
E888	8846972	Mitochondrial hepatopathy	32	1	15	47
E888	8849469	Mitochondrial diabetes	62	2	39	63
E888	8849470	Mitochondrial diabetes with eye problems	—	—	—	—
E888	8849471	Mitochondrial diabetes with ketoacidosis	—	—	—	—
E888	8849472	Mitochondrial diabetes with coma	—	—	—	—
E888	8849473	Mitochondrial diabetes with neurologic symptom	—	—	—	—
E888	8849474	Mitochondrial diabetes with renal complication	—	—	—	—
E888	8849475	Mitochondrial diabetes with multiple diabetic complications	—	—	—	—
E888	8849476	Mitochondrial diabetes without diabetic complication	—	—	—	—
E888	8849477	Mitochondrial diabetes with diabetic complication	—	—	—	—
E888	8849478	Mitochondrial diabetes with peripheral circulatory disorder	—	—	—	—
G318	8840933	Leigh syndrome	212	6	108	51
G318	8842457	Alpers' syndrome	—	—	—	—
G713	8841409	Mitochondrial myopathy	253	7	124	49
G713	8841410	Mitochondrial encephalomyopathy	1,786	49	932	52
H472	8848684	LHON	71	2	17	24
H494	8846059	CPEO	106	3	50	47
H498	8831018	Kearns-Sayre syndrome	19	1	10	53

CPEO, chronic progressive external ophthalmoplegia; ICD-10, International Statistical Classification of Diseases and Related Health Problems 10th Revision; LHON, Leber's hereditary optic neuropathy; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes; MERRF, myoclonus epilepsy associated with ragged-red fibers; MNGIE, mitochondrial neurogastrointestinal encephalopathy; PDHC, pyruvate dehydrogenase complex.
[a]This table shows the number of patients with mitochondrial diseases with each diagnosis categorized by domestic diagnosis codes for healthcare claims in Japan. According to the rules for publication of NDB data, we did not show the number of cases in categories with less than 10 patients (indicated by "—" in the table). The sum of patients is not equal to the total number of patients because some patients are given two or more diagnoses.

Table 3. Number of patients and prevalence of mitochondrial diseases in each prefecture in Japan from April 2018 to March 2019[a]

	NDB				Government report			
Prefecture	*n*	%	Prevalence	95% CI	*n*	%	Prevalence	95% CI
Hokkaido	169	5	3.2	2.7–3.7	62	4	1.2	0.9–1.5
Aomori	22	1	1.7	1.1–2.6	11	1	0.9	0.4–1.6
Iwate	43	1	3.5	2.5–4.7	18	1	1.5	0.9–2.3
Miyagi	79	2	3.4	2.7–4.3	29	2	1.3	0.8–1.8
Akita	19	1	1.9	1.2–3	8	1	0.8	0.4–1.6
Yamagata	29	1	2.7	1.8–3.8	13	1	1.2	0.6–2.0
Fukushima	33	1	1.8	1.2–2.5	20	1	1.1	0.7–1.7
Ibaraki	87	2	3.0	2.4–3.7	42	3	1.5	1.1–2.0
Tochigi	56	2	2.9	2.2–3.7	21	1	1.1	0.7–1.7
Gumma	54	1	2.8	2.1–3.6	26	2	1.3	0.9–2.0
Saitama	125	3	1.7	1.4–2	74	5	1.0	0.8–1.3
Chiba	162	4	2.6	2.2–3	64	4	1.0	0.8–1.3
Tokyo	477	13	3.5	3.2–3.8	182	12	1.3	1.1–1.5
Kanagawa	218	6	2.4	2.1–2.7	102	7	1.1	0.9–1.4
Niigata	56	2	2.5	1.9–3.2	28	2	1.2	0.8–1.8
Toyama	30	1	2.9	1.9–4.1	14	1	1.3	0.7–2.2
Ishikawa	36	1	3.1	2.2–4.4	15	1	1.3	0.7–2.2
Fukui	21	1	2.7	1.7–4.2	12	1	1.6	0.8–2.7
Yamanashi	13	1	1.6	0.8–2.7	1	1	0.1	0.1–6.8
Nagano	52	1	2.5	1.9–3.3	22	1	1.1	0.7–1.6
Gifu	44	1	2.2	1.6–3	16	1	0.8	0.5–1.3
Shizuoka	86	2	2.4	1.9–2.9	31	2	0.8	0.6–1.2
Aichi	174	5	2.3	2–2.7	52	3	0.7	0.5–0.9
Mie	39	1	2.2	1.6–3	11	1	0.6	0.3–1.1
Shiga	53	1	3.8	2.8–4.9	20	1	1.4	0.9–2.2
Kyoto	86	2	3.3	2.7–4.1	36	2	1.4	1.0–1.9
Osaka	263	7	3.0	2.6–3.4	115	8	1.3	1.1–1.6
Hyogo	162	4	3.0	2.5–3.5	63	4	1.1	0.9–1.5
Nara	44	1	3.3	2.4–4.4	19	1	1.4	0.9–2.2
Wakayama	29	1	3.1	2.1–4.5	15	1	1.6	0.9–2.7
Tottori	15	1	2.7	1.5–4.4	5	1	0.9	0.3–2.1
Shimane	22	1	3.2	2–4.9	13	1	1.9	1.0–3.3
Okayama	55	2	2.9	2.2–3.8	13	1	0.7	0.4–1.2
Hiroshima	73	2	2.6	2–3.3	34	2	1.2	0.8–1.7
Yamaguchi	24	1	1.8	1.1–2.6	13	1	0.9	0.5–1.6
Tokushima	16	1	2.2	1.2–3.5	7	1	1.0	0.4–2.0
Kagawa	19	1	2.0	1.2–3.1	10	1	1.0	0.5–1.9
Ehime	42	1	3.1	2.2–4.2	17	1	1.3	0.7–2.0
Kochi	18	1	2.5	1.5–4	3	1	0.4	0.1–1.2
Fukuoka	183	5	3.6	3.1–4.1	58	4	1.1	0.9–1.5
Saga	23	1	2.8	1.8–4.2	12	1	1.5	0.8–2.6
Nagasaki	50	1	3.7	2.8–4.9	26	2	1.9	1.3–2.8
Kumamoto	45	1	2.6	1.9–3.4	27	2	1.5	1.0–2.2
Oita	39	1	3.4	2.4–4.7	23	2	2.0	1.3–3.0
Miyazaki	40	1	3.7	2.6–5	17	1	1.6	0.9–2.5
Kagoshima	136	4	8.4	7.1–10	59	4	3.7	2.8–4.7
Okinawa	68	2	4.7	3.7–6	25	2	1.7	1.1–2.6
Japan	3,629		2.9	2.8–3	1,504		1.2	1.1–1.3

CI, confidence interval; NDB, National Database.

[a]This table shows the number of patients and the prevalence of mitochondrial diseases from April 2018 to March 2019 identified in this study. The same parameters reported by the government based on the number of patients using the Japanese medical expenses subsidy for intractable diseases including mitochondrial diseases are shown as a reference. Prefectures are listed according to their geographic location from north-east to south-west. % values do not sum to 100% because of rounding. Prevalence is prevalence per 100,000 population in each prefecture.

prefectures, at 56 and 59, respectively. Although it is important to consider the effect of population size in each prefecture, in Ishikawa and Okinawa, there were large differences in SPR by sex. The SPRs of female and male patients were 75 and 148.8 in Ishikawa, and 183.6 and 117.7 in Okinawa, respectively.

EBSPRs in each prefecture provided more conservative results than normal SPRs, indicating that values were reaching closer to 100. Similar to results for normal SPRs, Kagoshima and Okinawa had the highest EBSPRs of all prefectures, at 247.7 and 139.8, respectively. In contrast, Saitama and Fukushima had the lowest EBSPRs of all prefectures, at 61.8 and 71.3, respectively.

DISCUSSION

Using data from the Japanese NDB, we estimated that the number of patients with mitochondrial diseases from April 2018 through

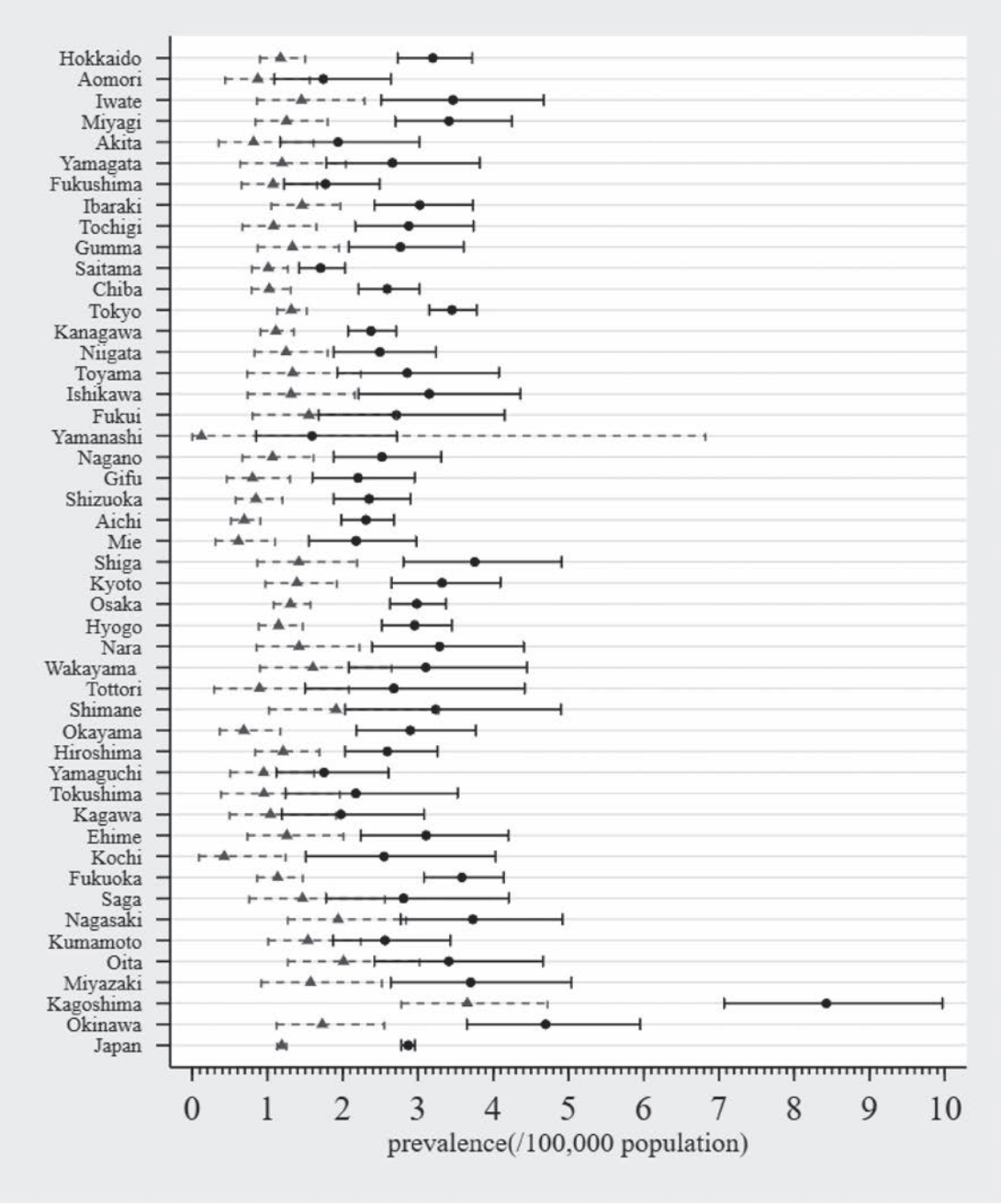

Figure 1. Estimated prevalence of mitochondrial diseases in each prefecture in Japan according to the NDB and government report. Prefectures in this figure are listed according to their geographic location from north-east to south-west. Black points represent the prevalence estimated by this study; solid black lines with caps on both ends represent 95% confidence intervals of the prevalence estimated by this study; grey triangles represent the prevalence indicated by the Japanese government; grey dashed lines with caps on both ends represent 95% confidence intervals of the prevalence indicated by the Japanese government.

March 2019 was 3,629, with a prevalence of 2.9 per 100,000 general population. This study is the first to comprehensively estimate the number of patients with mitochondrial diseases in Japan, along with the distribution of patients by sex, age, and geographic characteristics using health care claims data from the past 10 years.

The Japanese government has established a medical expense subsidy system for patients with intractable diseases, including mitochondrial diseases. According to government statistics from 2018,[28] 1,504 patients with mitochondrial diseases used this system. This number is less than half the number of patients with mitochondrial diseases identified in this study ($n = 3,629$). Similarly, the number and prevalence of patients in each prefecture identified as having mitochondrial diseases in this study were also greater than those using the subsidy system. However, it may not be appropriate to compare the number of patients identified in the present study with that in the government report. This is because, while certification for the government

Table 4. SPRs and EBSPRs of mitochondrial diseases in each prefecture in Japan from April 2018 to March 2019[a]

	Total				Female				Male			
Prefecture	*n*	SPR	95% CI	EBSPR	*n*	SPR	95% CI	EBSPR	*n*	SPR	95% CI	EBSPR
Hokkaido	169	114.3	97.7–132.9	113.2	96	119.7	96.9–146.1	116.9	73	107.6	84.3–135.3	106.6
Aomori	22	62.6	39.3–94.8	74.2	—	—	—	—	—	—	—	—
Iwate	43	124.1	89.8–167.1	117.4	23	125.1	79.3–187.8	114.7	20	122.8	75–189.6	112.5
Miyagi	79	119	94.3–148.4	115.9	43	123.0	89–165.6	116.6	36	114.5	80.2–158.5	110.3
Akita	19	71.4	43–111.5	82.2	—	—	—	—	—	—	—	—
Yamagata	29	95	63.6–136.4	97.2	15	92.4	51.7–152.4	96.5	14	97.7	53.4–163.9	100.1
Fukushima	33	62.7	43.2–88.1	71.3	18	66.0	39.1–104.2	78.0	15	59.4	33.3–98	76.1
Ibaraki	87	105.7	84.7–130.4	105.1	39	91.7	65.2–125.3	94.0	48	121.1	89.3–160.5	115.6
Tochigi	56	100.1	75.6–130	100.4	29	100.1	67–143.8	100.4	27	100.3	66.1–145.9	101
Gumma	54	96.9	72.8–126.5	97.9	28	96.6	64.2–139.6	98.1	26	97.6	63.8–143	99.3
Saitama	125	59	49.1–70.3	61.8	67	61.1	47.4–77.6	65.7	58	57	43.3–73.6	63.2
Chiba	162	90.2	76.9–105.3	91.1	76	81.4	64.1–101.8	84.0	86	100.1	80–123.6	100.4
Tokyo	477	119	108.6–130.2	118.4	258	122.7	108.2–138.6	121.3	219	115.1	100.3–131.4	114.1
Kanagawa	218	81.9	71.4–93.5	82.9	115	83.4	68.8–100	85.0	103	80.4	65.7–97.6	82.9
Niigata	56	88.7	67–115.2	91.2	31	93.0	63.2–132	95.4	25	84	54.3–124	90.4
Toyama	30	101.6	68.5–145	101.7	13	83.5	44.5–142.9	91.9	17	121.8	70.9–194.9	111.3
Ishikawa	36	109.8	76.9–152	107.3	13	75.0	39.9–128.2	86.7	23	148.8	94.3–223.3	125
Fukui	21	94.8	58.6–144.8	97.6	11	94.1	47–168.4	97.9	10	95.5	45.8–175.6	99.6
Yamanashi	13	56	29.8–95.8	73.8	—	—	—	—	—	—	—	—
Nagano	52	88.8	66.3–116.5	91.4	35	113.7	79.2–158.2	109.8	17	61.3	35.7–98.2	76.4
Gifu	44	76.9	55.9–103.3	82.0	25	82.6	53.4–121.9	88.5	19	70.6	42.5–110.2	82.4
Shizuoka	86	82.1	65.7–101.4	84.5	48	87.8	64.7–116.4	90.5	38	76	53.8–104.3	82.4
Aichi	174	78.6	67.3–91.2	80.0	97	84.8	68.8–103.5	86.6	77	72	56.8–90	76
Mie	39	76.1	54.1–104	81.8	19	70.4	42.4–109.9	81.0	20	82.5	50.4–127.5	90.4
Shiga	53	127.3	95.4–166.5	120.7	28	128.8	85.6–186.1	117.8	25	125.8	81.4–185.7	115.2
Kyoto	86	116.5	93.2–143.9	114.1	40	101.0	72.2–137.5	101.0	46	134.2	98.2–179	124
Osaka	263	103.9	91.7–117.3	103.8	136	100.4	84.2–118.8	100.5	127	107.8	89.9–128.3	107.1
Hyogo	162	102.9	87.7–120	102.8	77	90.9	71.8–113.6	92.4	85	116.6	93.1–144.1	114
Nara	44	116.1	84.4–155.9	112.1	19	92.0	55.4–143.6	95.7	25	144	93.2–212.5	123.9
Wakayama	29	110.6	74.1–158.9	107.5	15	105.6	59.1–174.2	103.4	14	116.3	63.6–195.2	108.2
Tottori	15	94	52.6–155	97.7	—	—	—	—	—	—	—	—
Shimane	22	115	72.1–174.1	109.3	10	98.9	47.5–182	100.2	12	133.5	69–233.2	113.4
Okayama	55	101.3	76.3–131.8	101.4	33	114.2	78.6–160.4	109.9	22	86.5	54.2–130.9	92.6
Hiroshima	73	89.9	70.5–113.1	91.7	34	79.2	54.8–110.7	84.6	39	101.9	72.4–139.3	102
Yamaguchi	24	62.7	40.2–93.3	73.5	11	53.4	26.7–95.6	72.9	13	73.2	39–125.1	87
Tokushima	16	77.9	44.6–126.6	87.9	—	—	—	—	—	—	—	—
Kagawa	19	69.6	41.9–108.7	80.8	—	—	—	—	—	—	—	—
Ehime	42	110.4	79.6–149.2	108.0	23	112.2	71.1–168.3	107.7	19	108.3	65.2–169	105.4
Kochi	18	92.1	54.6–145.6	96.3	—	—	—	—	—	—	—	—
Fukuoka	183	123.3	106.1–142.5	121.4	99	123.5	100.4–150.4	120.2	84	122.7	97.9–151.9	118.8
Saga	23	96.9	61.4–145.4	98.8	12	94.2	48.7–164.5	97.8	11	100.6	50.2–180	101.6
Nagasaki	50	131.2	97.4–172.9	123.0	25	121.2	78.5–179	113.0	25	142.5	92.2–210.3	123.2
Kumamoto	45	89	64.9–119.1	91.9	20	73.2	44.7–113	82.7	25	107.4	69.5–158.5	105.3
Oita	39	120.7	85.8–164.9	114.8	25	143.6	92.9–212	124.5	14	93.6	51.2–157.2	98.1
Miyazaki	40	129.1	92.2–175.8	120.3	19	113.2	68.2–176.8	107.7	21	147.5	91.3–225.4	123.4
Kagoshima	136	294	246.6–347.7	247.7	79	314.2	248.8–391.6	237.3	57	268	203–347.3	196.5
Okinawa	68	152.3	118.3–193.1	139.8	43	183.6	132.9–247.3	152.4	25	117.7	76.2–173.8	111
Japan	3,629	100	96.8–103.3	100	1,917	100.0	95.6–104.6	100	1,712	100	95.3–104.9	100

CI, confidence interval of SPR; EBSPR, empirical Bayes estimator of standardized prevalence ratio; SPR, standardized prevalence ratio.
[a]This table shows SPRs and EBSPRs of patients with mitochondrial diseases in each prefecture from April 2018 to March 2019 identified in this study. SPRs and EBSPRs were calculated for total, female and male patients. According to the rules for publication of NDB data, we did not show the number of cases in categories with less than 10 patients (indicated by "—" in the table). Values were also concealed when the number of either of males or females was less than 10.

subsidy system is typically based on Japanese clinical criteria,[29] some patients with relatively mild disease severity may not use this system, whose main purpose is to provide treatment-related financial support to patients. Therefore, it may be that the government-reported number of patients will inevitably be an underestimate compared to that identified in the present study.

A previous study in Japan reported that 233 patients had MELAS, with a prevalence of 0.18 (95% CI, 0.17–0.19) per 100,000 general population.[18] In this study, we identified 284 patients with MELAS, with a prevalence of 0.22 (95% CI, 0.20–0.25) per 100,000 general population. Therefore, the number of patients and prevalence of MELAS identified in this study are comparable to those of the previous study. We expect that this epidemiological information will contribute to improving the health care system for patients with mitochondrial diseases in Japan.

We found that prevalence of mitochondrial diseases differed among prefectures. While the reasons for this are unclear, previous studies from other countries suggest that prevalence may differ by geography.[4,6,11,19] For example, a study conducted in the northeast of England reported that patients showing clinical manifestations had a prevalence of 9.6 per 100,000 adult general population,[6] which is about three times higher than that found in this study (2.9 per 100,000 general population). Moreover, a study in Finland suggested that geographical and cultural isolation may cause differences in prevalence.[11,19] Furthermore, a study in Australia showed that the prevalence of mitochondrial diseases among children whose mothers were born in Lebanon is much higher than that for mothers born in other countries.[12] However, we were careful when comparing results obtained using different methods for two main reasons. First, prevalence estimated by a single expert clinical and laboratory referral center can be a greater overestimation than that determined by a study using national health database with large samples. Second, patients extracted from NDB differ in diagnosis period. This difference may affect the number of patients between studies because clinical criteria and the diagnostic methods applied depend on the diagnosis period.[30]

To our knowledge, however, no meaningful report from Asian countries has appeared to date. Further studies are needed to examine the prevalence of mitochondrial diseases in Asia and to compare the results obtained in this study with those in other Asian countries.

We found differences in the prevalence of mitochondrial diseases among prefectures, despite the fact that the genetic background of the Japanese population is thought to be relatively uniform across the country. In addition to genetic factors, the variability in prevalence may be related to differences in health care infrastructure among prefectures in Japan. That is, there may be a concentration of patients in specific medical facilities that are well equipped for diagnosis and treatment. For example, within the Tokyo metropolitan area, the number of patients and prevalence of mitochondrial diseases in Saitama (125, 1.7/100,000), a densely populated prefecture with a population of seven million, was lower than in other large prefectures like Chiba (162, 2.6/100,000) and Kanagawa (218, 2.4/100,000), which are also adjacent to Tokyo. We speculate that there are two main reasons for this. First, there are fewer physicians and specialists per prefecture population in Saitama than Chiba and Kanagawa. Additionally, there are few large hospitals with the ability to provide care for patients with mitochondrial diseases. Second, residents of Saitama have easy access to large hospitals in Tokyo. A similar phenomenon is thought to be occurring in local cities outside the metropolitan area. To improve the provision of health care for patients with mitochondrial diseases, we suggest that, in addition to training healthcare workers and improving clinical guidelines, there is a need to increase the number of medical facilities with the competency to provide care for patients with mitochondrial diseases. Additionally, geographic factors may also be important. We found that patients in Kagoshima and Okinawa had higher prevalence, SPRs, and EBSPRs than all other prefectures. While the reason for the higher values is unclear, these findings suggest that there may be geographic effects because Kagoshima and Okinawa are located in the southernmost part of Japan.

This study has several limitations. First, it is possible that some patients included in this study did not actually have mitochondrial diseases. While a definitive diagnosis of mitochondrial diseases requires an integrated approach, including genetic testing, some of these tests are not covered by the current health insurance system in Japan, and the number of medical facilities with the capacity to perform them is limited. Therefore, it is difficult to determine whether the diagnoses used in this study are definitive of mitochondrial diseases. However, we expect that few patients would have been misdiagnosed because, unlike common or frequent diseases, mitochondrial diseases are carefully and strictly diagnosed by clinicians. Second, due to the nature of the NDB, patients can be duplicated if they or their caregivers change their health insurance scheme due to a job change, unemployment, or employment, thus leading to a potential overestimation of cases. A previous study using the NDB examined the effects of changing health insurance schemes on the estimation of prevalence.[31] Using the method described in this previous study,[31] we calculated the maximum impact of changing health insurance schemes to be about 6.6% by summing the proportion of patients who were newly unemployed within a year (1.7%) and the proportion who underwent a job change (4.9%) in 2018.[32] Therefore, the effect of changing health insurance schemes is likely relatively small. Third, information on patients who receive public assistance is not included in the NDB. The proportion of people who received public assistance in Japan was about 1.65% in February 2019.[33] Thus, this is expected to have caused only a small underestimation. Despite the above-mentioned limitations, our study provides a valid estimation of the number of patients and prevalence of mitochondrial diseases in Japan.

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Long-Term Progression and Rapid Decline in Hearing Loss in Patients with a Point Mutation at Nucleotide 3243 of the Mitochondrial DNA

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Abstract: Patients with m.3243A>G mutation of mitochondrial DNA develop bilaterally symmetric sensorineural hearing loss. However, it is unclear how fast their hearing loss progresses over time, and whether they experience rapid progression of hearing loss. In the present study, we conducted a long-term hearing evaluation in patients with MELAS or MIDD who harbored the m.3243A>G mutation of mitochondrial DNA. A retrospective chart review was performed on 15 patients with this mutation who underwent pure-tone audiometry at least once a year for more than two years. The mean follow-up period was 12.8 years. The mean progression rate of hearing loss was 5.5 dB per year. Hearing loss progressed rapidly to be profoundly deaf in seven patients during the observation period. Heteroplasmy and age-corrected heteroplasmy levels correlated with the age of onset of hearing loss. These results indicate that patients with m.3243A>G mutation have a gradual progression of hearing loss in the early stages and rapid decline in hearing to be profoundly deaf in approximately half of the patients. Although it is possible to predict the age of onset of hearing loss from heteroplasmy and age-corrected heteroplasmy levels, it is difficult to predict whether and when the rapid hearing loss will occur.

Keywords: hearing loss; dizziness; disequilibrium; diabetes; mitochondrial gene mutations

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1. Introduction

Sensorineural hearing loss is frequently associated with mitochondrial disease; it is observed in approximately half of the patients with three main syndromes—chronic progressive external ophthalmoplegia (CPEO); myoclonus epilepsy associated with ragged-red fibers (MERRF); mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) [1]. Hearing loss mainly involves the cochlea in mitochondrial diseases, but it sometimes accompanies central auditory abnormalities [1–5].

Mitochondrial diseases are categorized into two groups: those characterized by ragged-red fibers (RRF), including MELAS, MERRF, and CPEO, and those caused by mutations in protein-coding genes, such as pure encephalopathy without RRF. RRF is a characteristic microscopic appearance of the muscle stained with Gomori trichrome, which is due to the accumulation of abnormal mitochondria below the plasma membrane of the muscle fiber. Hearing loss is highly prevalent in the first group but rarely seen in the second group. Oxidative phosphorylation is impaired in both types of encephalopathy; however, mitochondrial protein synthesis is impaired only in the first group, suggesting that hearing loss is caused by impairment of protein synthesis similar to RRF.

An A-to-g transition mutation at nucleotide pair (np) 3243 in mitochondrial DNA (mtDNA) has been documented in most patients with MELAS and a few patients with CPEO. This mutation has been identified in pedigrees with maternal transmission of diabetes and deafness among families of different racial backgrounds [6–10]. It has been reported that patients with higher heteroplasmy levels of this point mutation exhibit MELAS [11,12], while those with low heteroplasmy levels mainly develop hearing loss and diabetes; those with a low heteroplasmy level have maternally inherited diabetes and deafness (MIDD) [9].

In patients with MELAS and MIDD, hearing is generally normal at birth, and hearing loss occurs eventually, appearing as early as in the teens or twenties and as late as the fifties. Hearing loss, in general, is bilateral and symmetrical; a pure-tone audiogram shows a flat type or falling type of sensorineural loss bilaterally in most cases [6,9]. Hearing loss mainly involves the cochlea [6,13,14]; however, when the disease progresses, retro-labyrinthine and central auditory pathways are also affected [15,16]. In addition to hearing loss, the vestibular system is also involved, and patients eventually suffer from impairment of balance and gait [6,17–19].

We previously reported that, when present for several years, patients with np3243 point mutation showed a slightly progressive decline in their hearing; the progression rate of hearing loss ranged from 1.5 to 7.9 dB per year [7]. However, it is unknown how rapidly their hearing loss progresses during the long-term period, and whether there is a rapid progression of hearing loss. Therefore, in the present study, we evaluated their hearing in the long-term period in patients with MELAS or MIDD who harbored m.3243A>G mutation of mtDNA. We also investigated the relationship between heteroplasmy and age-corrected heteroplasmy levels and the age of onset of hearing loss and the progression rate of hearing loss, as well as the relationship between the age of onset of hearing loss, diabetes, and balance disorder.

2. Materials and Methods

2.1. Patients

In total, 27 patients with hearing loss and an A-to-g transition at np 3243 in the mtDNA visited the Department of Otolaryngology and Head and Neck Surgery, at the University of Tokyo Hospital, between 1989 and 2021. Among them, 15 patients who underwent audiological examinations for more than two years or until they became completely deaf were enrolled. The patients consisted of four males and eleven females, and their ages ranged from 22 to 66 years (mean: 40 years) at their first visit to our clinic. All patients had hearing loss, with a pure-tone average (PTA) value being greater than 25 dB HL (hearing level) on the initial audiological examination. They were interviewed regarding the onset of hearing loss, balance–gait disorder, diabetes mellitus, and the presence of other signs and symptoms. The presence of diabetes mellitus was confirmed by doctors in the Department of Nutrition and Metabolism of our hospital or their affiliated hospital, and the blood glucose and HbA1c levels were periodically measured in all patients. All procedures were in accordance with the Helsinki declaration and were approved by the University of Tokyo Human Ethics Committee (No. 2487). All patients gave informed consent for the use of their clinical data.

2.2. Audiological and Neuro-Otological Evaluation

The patients were evaluated using pure-tone audiometry, in general, every three months, to assess the longitudinal changes in their hearing thresholds. The pure-tone average (PTA) values were calculated as the mean air conduction threshold at 0.5, 1, 2, and 3 kHz. The pure-tone threshold at 3 kHz was obtained from the mean of 2 and 4 kHz, as it is not routinely measured in Japan. For calculating the PTA values, the hearing thresholds at the frequencies showing off-scale were calculated as 5 dB over the maximum sound level generated by an audiometer. The patients were also evaluated using speech

recognition test, tympanometry, acoustic reflex threshold test, auditory brainstem response, and distortion-product otoacoustic emissions (DPOAEs), when necessary.

The patients also underwent a battery of neuro-otological evaluations consisting of a physical examination, neurological examination, and neuro-otological examinations, including caloric and cervical vestibular evoked myogenic potential (cVEMP) testing.

Caloric testing was performed with 2 mL ice water irrigation of the external auditory canal for 20 s. This caloric stimulation method is easier to perform than bithermal irrigation and has high sensitivity and specificity for detecting canal paresis [20]. Electronystagmography was used to record the induced nystagmus while the subject lay supine with their head raised at an angle of 30 degrees. The percentage of canal paresis was calculated as $100 \times |(\text{MSEVr} - \text{MSEVl})/(\text{MSEVr} + \text{MSEVl})|$, where MSEVr is the maximum slow-phase eye velocity of the right side, and MSEVl is that of the left side. A value of canal paresis >20% was regarded as abnormally reduced on the affected side [21]. When MSEVr and MSEVl were <10 degrees/s, it was regarded as a reduced response on both sides [22].

In the role of a stimulus for cVEMP, short tone bursts of 500 Hz (95 dB normal hearing level; 135 dB SPL (peak value); rise/fall time, 1 ms; plateau time, 2 ms) were presented through headphones (type DR-531; Elega Acoustic Ltd., Tokyo, Japan). Surface electromyographic activity was recorded in supine patients from symmetrical sites over the upper half of each sternocleidomastoid muscle (SCM), with a reference electrode on the lateral end of the upper sternum. During recording, the patients were instructed to raise their heads slightly to continuously contract the SCM. Background EMG was monitored during recording to confirm that subjects maintained SCM activity at a sufficient level (150 μV). We analyzed the first biphasic wave (p13-n23) from the ipsilateral SCM to the stimulated side. For the evaluation of amplitude. The percentage of cVEMP asymmetry ratio (cVEMP AR) was calculated as $100|(\text{Ar} - \text{Al})/(\text{Ar} + \text{Al})|$, where Ar is the amplitude of p13-n23 on the right side, and Al is the amplitude of the p13-n23 on the left side. On the basis of results from normal subjects, the upper limit of cVEMP AR was set to 34.0 [23]. We regarded it as an "absent" response when no reproducible p13-n23 was present in two runs. We regarded it as a "reduced" response when a reproducible p13-n23 was present, and the AR was greater than the predefined upper limit for normal subjects. We regarded it as bilaterally abnormal responses when the response was absent on both sides.

2.3. DNA Studies

The invader assay contract by BML (Tokyo, Japan) was applied for the screening of mitochondrial tRNA (Leu). Briefly, mtDNA isolated from the peripheral leukocytes of patients and 1.2 μL of primary probe/invader oligonucleotides mixture (containing 0.5 umol/L wild-type primary probes, 0.5 umol/L mutant primary probes, 0.05 umol/L invader oligonucleotide, and 10 mmol/L 3-(N-morpholino) propanesulfonic acid) were poured into each well of the plates. Fluorescent resonance energy transfer (FRET)/Cleavase mixture (Hologic, Marlborough, MA, USA) was added to the probe/invader oligonucleotide-containing plates. Subsequently, 3 μL of 5–100 mol/L synthetic target oligonucleotides (positive control), 10 ug/mL yeast tRNA (no target control), and denatured genomic DNA samples (>15 ng/μL) were added. Next, 6 μL of mineral oil (Sigma, St. Louis, MO, USA) was overlayed into all reaction wells and incubated at 63 °C for 4 h. After incubation, the fluorescence was measured using a Cyto Fluor 4000 fluorescent microplate reader. The heteroplasmy rate for mitochondrial mutations was quantified by the detection of fluorescently labeled and digested PCR products through a fluorescence imaging system [24,25]. The age-corrected heteroplasmy level in leucocytes was calculated using the following formula: (leucocyte heteroplasmy)/$0.977^{(\text{age}+12)}$. This correction was previously published by Grady et al. [26]. The current paper refers to this value as the age-corrected heteroplasmy level in leucocytes.

2.4. Statistical Analysis

The correlation coefficients were calculated to assess the relationship between the heteroplasmy and age-corrected heteroplasmy levels and the progression rate of hearing loss and between the heteroplasmy and age-corrected heteroplasmy levels and the onset of hearing loss, balance or gait disorder, and diabetes mellitus, using JMP 16 (SAS Institute, Cary, NC, USA). The age of onset of hearing loss, diabetes, and balance disorder was compared against each other using the Tukey–Kramer test. We performed the Shapiro–Wilk W test to value the normality of the sample. The relationship between the two continuous variables was analyzed by single regression analysis. R2 represented the coefficient of determination, and the significance of the slope indicated the *p*-value. $p < 0.05$ was considered statistically significant.

3. Results

The patients' demographic characteristics of the patients are shown in Table 1. The heteroplasmy levels ranged from 3% to 37%, with the mean being 23.9%. In terms of gender, 4 patients were male, and 11 were female. Most patients had bilaterally symmetric hearing loss with a horizontal or sloping type of audiogram. The mean age of onset of hearing loss was 28.6 years, with acquired hearing loss being perceived as early as 10 years of age in the earliest cases and as late as 56 years of age in the latest cases. At their first visit, 13 patients had diabetes mellitus, 3 had cerebellar atrophy or stroke on head MRI, and 1 had a cardiac disease; two patients eventually developed diabetes during the follow-up period. Other than hearing loss and diabetes mellitus, 11 patients did not show any symptoms or signs suggestive of MELAS.

The observation period ranged from 2 to 22 years, with a mean of 12.8 years. Hearing loss progressed in all patients during the observation period (Figures 1 and 2, Supplementary Figures S1 and S2). In seven patients, hearing deteriorated rapidly to complete deafness from 40 to 63 years of age (mean: 50 years). This episode was not associated with the worsening of pre-existing signs such as diabetes or other symptoms or signs. Before the rapid progression of hearing loss, their hearing level ranged from 56 to 80 dB HL. The rapid progression occurred on both ears almost simultaneously in three patients and at different times in two patients, with the periods between the ears being 1 and 7 years, respectively, and only on one ear in two patients (Figure 3 and Supplementary Figure S3). Although all these patients were treated with oral or systemic steroids, their hearing did not show any improvement. The heteroplasmy level ranged from 9% to 30% (mean: 20%) in seven patients, who showed rapid progression of hearing loss, and from 3% to 37% (mean: 23%) in the remaining eight patients, with no significant difference between them. The age-corrected heteroplasmy level ranged from 18.4% to 95.7% (mean: 68.6%) in seven patients, who showed rapid progression of hearing loss, and from 20.2% to 81.6% (mean: 64.4%) in the remaining eight patients, with no significant difference between them.

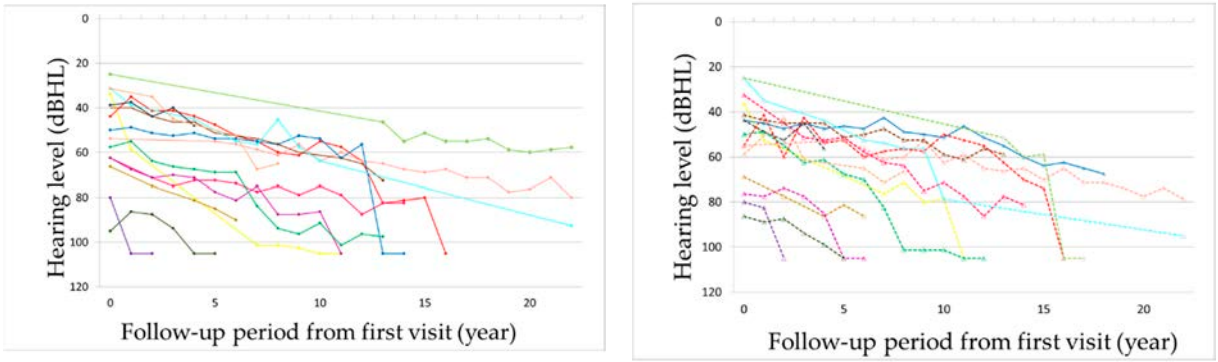
The mean rate of the progression of hearing loss in all 15 patients was 5.5 dB per year. In eight patients, who did not show rapid deterioration of hearing, the progression rate of hearing loss was 3.0 dB per year, while it was 1.9 dB per year before the rapid deterioration in seven patients. When the progression rate of hearing loss prior to the rapid deterioration in the latter was added to the calculation, the mean progression rate of hearing loss was 2.5 dB per year in all patients. When the progression rate of hearing loss prior to the rapid deterioration in the latter was added to the calculation, the mean progression rate of hearing loss was 2.5 dB per year in all 15 patients and did not differ significantly between the patients with and without rapid deterioration in hearing.

Caloric and cVEMP tests were performed in 13 out of 15 patients. In the caloric test, 4 (30%) out of 13 patients showed unilaterally decreased response, and 6 (46%) showed bilaterally decreased response. In cVEMP, 6 (46%) out of 13 patients showed unilateral abnormalities, and 7 (54%) showed bilateral abnormalities.

Table 1. Demographic characteristics of the patients.

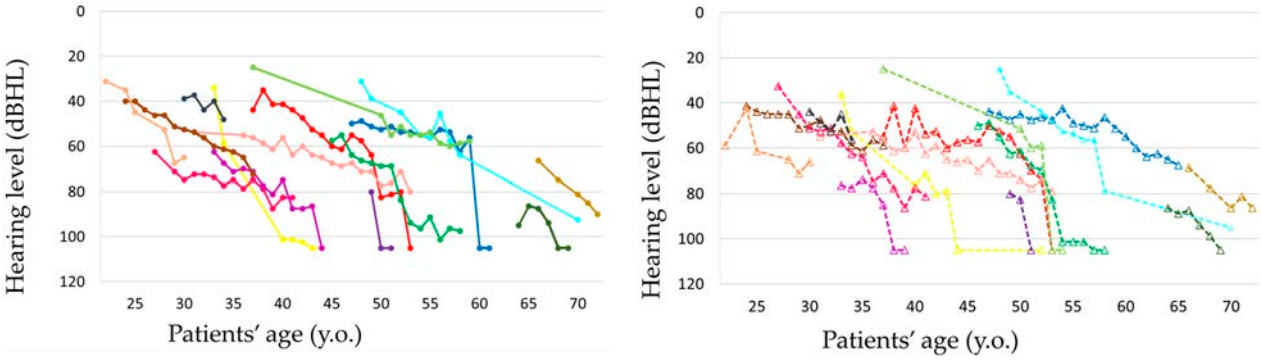
Heteroplasmy Level	Age-Corrected Hetero-Plasmy Level	Gender	Onset of Hearing Loss (y.o.)	Right HL at First Visit (dBHL)	Left HL at First Visit (dBHL)	Progression Rate of Hearing Loss (dB/yr)	Rapid Decline of Hearing	CI	Onset of Balance Disorder (y.o.)	Caloric Test	cVEMP	Onset of Diabetes (y.o.)	MIDD/MELAS
37	81.6	F	15	31.3	58.8	4.2				NE	NE	24	MELAS
36	95.7	M	15	38.8	43.8	2.5		y	31	UNI-H	UNI-N.R.	22	MIDD
32	74.0	F	15	40	41.3	2.5			24	NOR	UNI-N.R.	22	MIDD
30	93.8	F	32	43.8	55	4.8	y	y	54	NOR	UNI-N.R.	39	MIDD
30	81.6	F	30	53.8	55	1.1			33	UNI-H	BI-N.R.	10	MIDD
29	82.6	F	31	33.8	36.3	9.7	y	y	31	UNI-H	UNI-N.R.	21	MIDD
29	71.9	F	22	62.5	32.5	2.1			13	BI-H	BI-N.R.	26	MIDD
25	71.2	F	15	62.5	76.3	6.6	y		30	BI-H	BI-N.R.	21	MELAS
21	81.0	F	45	57.5	50	4	y	y	45	BI-H	BI-N.R.	45	MIDD
15	62.0	F	10	80	80	26.5	y	y		BI-H	BI-N.R.	26	MIDD
14	82.1	M	30	95	86.3	4.5		y	61	UNI-H	UNI-N.R.	33	MELAS
14	43.8	M	37	25	25	2.3	y			NOR	BI-N.R.	31	MELAS
9	35.5	F	30	50	43.8	5	y		47	BI-H	BI-N.R.	26	MIDD
5	20.2	M	46	31.3	25	3.25				NE	NE	20	MIDD
3	18.4	F	56	66.3	68.8	3.95				BI-H	UNI-N.R.	47	MIDD

BI-H: bilateral hyporeflexia; BI-N.R.: bilateral no-response; CI: cochlear implantation; cVEMP: cervical vestibular-evoked myogenic potential; F: female; HL: hearing level; M: male; MELAS: mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MIDD: maternally inherited diabetes and deafness; NE: not examined; NOR: normal; UNI-H: unilateral hyporeflexia; UNI-N.R.: unilateral hyporeflexia; y: yes; y.o.: years old; yr: year.



Right ear Left ear

Figure 1. Chronological progression of hearing level from the first visit. The different color indicates the different patient, and the same color indicates the same patient.



Right ear Left ear

Figure 2. Relationship between patients' age and progression of their hearing loss. The different color indicates the different patient, and the same color indicates the same patient.

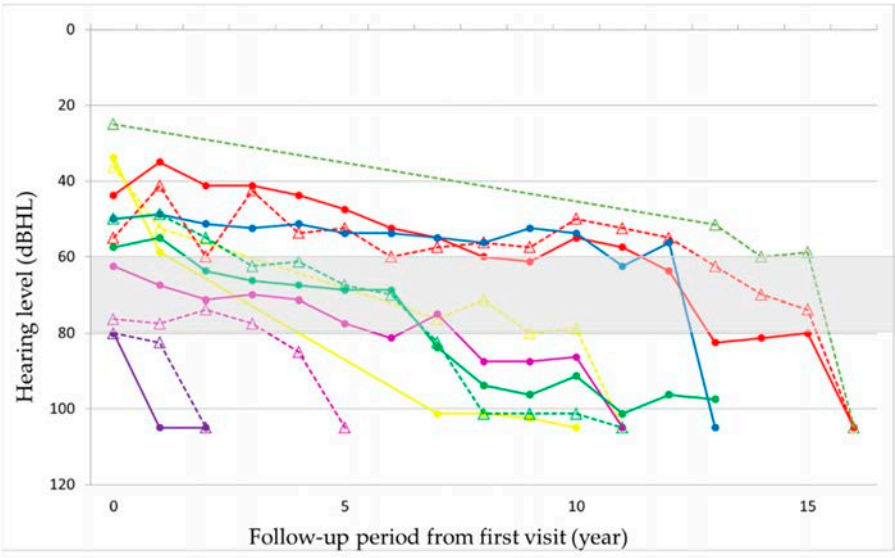


Figure 3. Chronological change in hearing level from the first visit in patients who showed a rapid decline in hearing. The different color indicates the different patient, and the same color indicates the same patient. The solid lines with the shaped mark: right ear. The dashed line with the triangle mark: left ear. Two patients (light green and blue) only have one ear shown because the rapid decline was only seen in one ear.

The mean age of onset of hearing loss, diabetes, and balance disorder was 28.6, 27.5, and 36.9 years, respectively (Figure 4). Based on the Shapiro–Wilk test, the *p*-values for the age of onset of hearing loss, diabetes, and balance disorder were 0.269, 0.400, and 0.189. The onset of balance disorder was delayed, compared with hearing loss and diabetes, but the difference was not statistically significant. However, balance disorder did not manifest in five patients until the end of the observation period; if we assume that the balance disorder appeared in these five patients one year later than the end of the observation period, the difference would be statistically significant ($p = 0.0092$ vs. diabetes; $p = 0.0161$ vs. hearing loss).

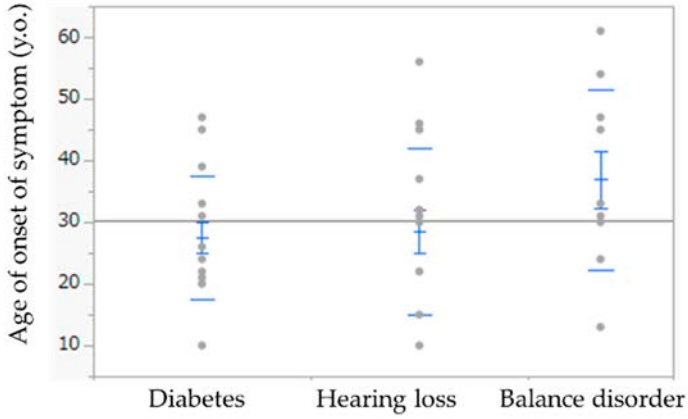
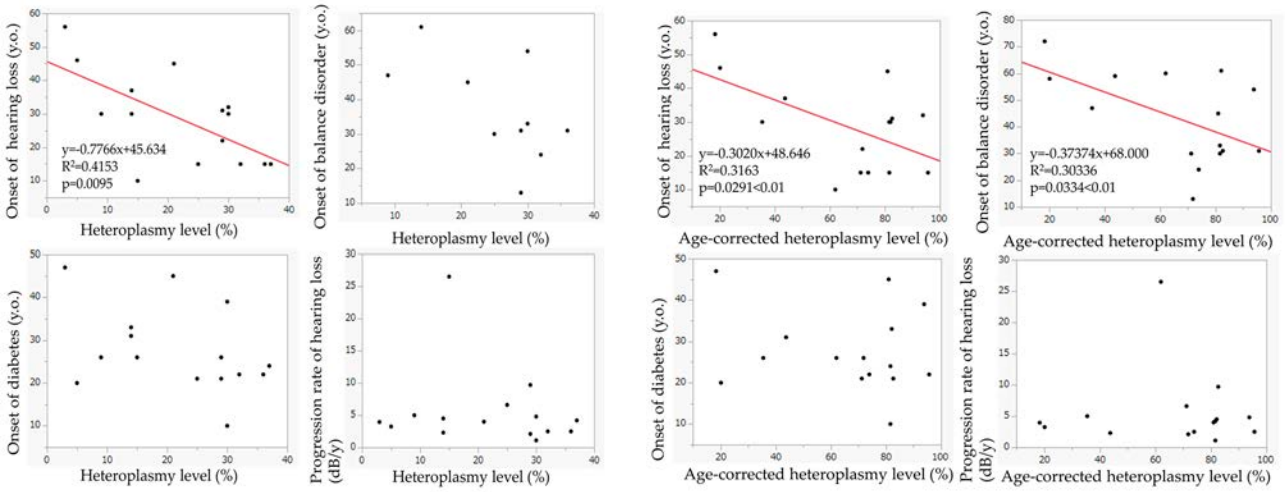


Figure 4. Age of the onset of diabetes, hearing loss, and balance disorder. Blue upper long bar, upper whisker; blue upper short bar, upper quartile; blue center short bar, median; blue lower short bar, lower quartile; blue lower long bar, lower whisker.

Figure 5a shows the relationship between the heteroplasmy level and the ages of onset of hearing loss, diabetes mellitus, and balance–gait disorder and between the heteroplasmy levels and the progression rate of hearing loss. The heteroplasmy levels showed a significant relationship with the onset of hearing loss ($p = 0.0095$); hearing loss appeared at a younger age in patients with higher heteroplasmy levels. Such a trend was also observed in the relationship between the heteroplasmy levels and the onset of balance disorder. The heteroplasmy levels were not associated with the onset of diabetes or the progression rate of hearing loss.

Figure 5b shows the relationship between the age-corrected heteroplasmy level and the ages of onset of hearing loss, diabetes mellitus, and balance–gait disorder, and between the heteroplasmy level and the progression rate of hearing loss. Age-corrected heteroplasmy levels showed significant correlations with the onset of hearing loss ($p = 0.0291$) and the onset of balance disorder ($p = 0.0334$); balance disorder appeared at a younger age in patients with higher age-corrected heteroplasmy. Age-corrected heteroplasmy level was not associated with the onset of diabetes or the progression rate of hearing loss.



(**a**) (**b**)

Figure 5. (**a**) Relationship between heteroplasmy level and the onset of hearing loss, balance–gait disorders, and diabetes mellitus and the progression rate of hearing loss; (**b**) relationship between age-corrected heteroplasmy level and the onset of hearing loss, balance disorders, and diabetes mellitus and the progression rate of hearing loss. The red lines: the regression lines.

4. Discussion

The current study evaluated the hearing in 15 patients with m.3243A>G mutation of mtDNA in the long-term period from 2 to 26 years (mean: 12.8 years) after their first hearing test. The mean age of onset of hearing loss was 28.6 years; hearing loss occurred between 10 and 56 years old. The age of onset of hearing loss was correlated with the heteroplasmy and age-corrected heteroplasmy levels. Initially, from the start of their follow-up, their hearing loss progressed gradually, but the hearing loss progressed rapidly to deafness in seven patients during the observation period. The hearing level ranged from 56 to 80 dB HL before the rapid deterioration of hearing. The progression rate of hearing loss before the rapid deterioration in these patients did not significantly differ from that in the remaining eight patients who did not show rapid hearing deterioration. All these results indicate that it is difficult to predict the rapid hearing decline in patients with m.3243A>G mutation of mtDNA. Oral or systemic steroid treatment was not effective in improving hearing loss.

Unlike m.1555A>G mutation, hearing loss caused by m.3243A>G mutation of mtDNA is progressive. When we first reported the audiological findings of five patients with this mutation in 1996, the progression rate of hearing loss ranged from 1.5 to 7.9 dB per year [8]. Since other studies have not reported the progression rate of hearing loss, it has not been unclear how rapidly the hearing declines in patients with the m.3243A>G mutation. In the current study, the progression rate of hearing loss ranged from 1.1 to 26.5 dB per year, with a mean of 5.5 dB per year. The progression rate of hearing loss was 3.0 dB per year in eight patients without rapid deterioration of hearing and 1.9 dB per year prior to the rapid deterioration in the remaining seven patients. When only the progression rate of hearing loss prior to the rapid deterioration in the latter was added in the calculation, the mean progression rate of hearing loss was 2.5 dB per year in all patients. This progression rate of hearing loss is more rapid than that seen in elderly subjects with age-related hearing loss. Therefore, patients with m.3243A>G mutation should be informed that their hearing loss will progress by an average of 25 dB after 10 years and advised to start wearing hearing aids at an early stage and structure their future living environment.

In the current study, the rapid progression of hearing loss occurred in 7 out of 15 patients, in both ears almost simultaneously in 3 patients, at different times in 2 patients, and only on one ear in 2 patients. This finding is quite interesting since such a rapid decline in hearing has not been reported except in one study. Oshima et al. (1996) reported

the case of a 35-year-old woman with a complaint of right hearing loss and tinnitus in whom the pure-tone audiogram demonstrated 40 dB flat-type sensorineural hearing loss on the left and 85 dB saucer-type sensorineural hearing loss on the right ear [9]. Although the hearing at middle frequencies on the right ear improved after oral administration of steroids, it fluctuated and did not respond to oral administration of steroids or glycerol thereafter. Since hearing did not improve by oral or systemic administration of steroids in any of our patients, the pathophysiology of the fluctuating hearing in the case reported by Oshima et al. [9] is likely different from that of rapid hearing progression in our patients.

In our case series, the rapid decline in hearing occurred after the hearing loss exceeded 55 dB HL. Prior to the rapid progression of hearing loss, their hearing level ranged from 56 to 80 dB HL. It is unclear why such a rapid decrease in hearing occurred. Since the rapid decline in patients' hearing was not associated with worsening of the pre-existing signs and symptoms or other signs and symptoms, it is unlikely that it was caused by a rapid decline in systemic mitochondrial function, at least in the four patients who had rapid hearing loss in both ears at different times or only in one ear. It is possible that the vascular supply to the cochlea was compromised due to diabetes mellitus or the stroke-like episode seen in MELAS. It is also possible that the endolymphatic potential was rapidly reduced due to the acute impairment of energy production in the stria vascularis.

Human temporal bone histopathological studies in patients with MIDD and MELAS showed that the stria vascularis most severely degenerated [27–29]; in a patient with MIDD, there was marked degeneration of the stria vascularis and outer hair cells throughout the cochlea, as well as a reduction in the number of spiral ganglion cells at the base [27]. Severe degeneration of the stria vascularis and degenerative change in the spiral ganglion cells were observed in two patients with MELAS, in whom quantitative DNA studies showed that the proportion of mutant to wild-type mtDNA was similar in both histologically affected and unaffected tissues within the inner ear. Long-term administration of germanium dioxide causes renal failure, emaciation, and muscle weakness in humans [30,31] and body weight loss, myopathy, and nephropathy in rats. The skeletal muscles of rats treated with germanium dioxide showed numerous ragged-red fibers, cytochrome c oxidase-deficient fibers, and the accumulation of electron-dense material in the mitochondria [30,32,33], which resembles the pathological findings observed in patients with mitochondrial encephalomyopathy. We previously reported that guinea pigs fed chows containing 0.5% germanium dioxide for 2 months developed hearing loss, mainly due to the degeneration of the stria vascularis and cochlear supporting cells, and exhibited decreased cytochrome c oxidase activity in the skeletal muscles and kidney [34]. No apparent pathological changes were observed in the utricle, semicircular canal, or the cochlear or vestibular nerve fibers, indicating that germanium dioxide-induced mitochondrial dysfunction mainly affects the stria vascularis and supporting cells in the cochlea, as in the skeletal muscles and kidney, causing hearing impairment in the guinea pigs. This animal study also supported the importance of mitochondrial function in the stria vascularis for the maintenance of hearing function.

In the present study, patients noted balance–gait disorder later, compared with hearing loss, and five patients were not aware of balance or gait disorder. Balance–gait disorder tended to appear later, compared with hearing loss and diabetes mellitus. The use of cVEMP is essential in the diagnosis of saccular dysfunction in patients with moderate-to-profound sensorineural hearing loss [35]. The low metabolic rate of the vestibular apparatus, compared with that of the stria vascularis, may make the vestibule more resistant to mtDNA mutations, leading to a later onset of vestibular dysfunction [21]. However, in 13 patients who underwent caloric and cVEMP tests, 4 (31%) and 6 patients (46%) showed decreased response unilaterally and bilaterally, respectively, in the caloric test, and 6 (46%) and 7 patients (54%) showed abnormalities unilaterally and bilaterally, respectively, in the cVEMP test. The caloric test evaluates the function of the lateral semicircular canal and the superior vestibular nerve, and the cVEMP evaluates the function of the saccule and the inferior vestibular nerve. Therefore, the vestibular systems, semicircular canals, and

otolith organs are also frequently involved in patients with m.3243A>G mutation. Balance and gait disturbances may not become apparent until the vestibular function is severely impaired. It is interesting to note that seven patients with m.3243A>G mutation who had abnormal findings in the caloric and cVEMP tests showed normal responses in the galvanic VEMP test [18], which indicates that the peripheral vestibular end-organs are primarily affected similarly to the auditory system.

Human temporal bone histopathological studies on MIDD and MELAS demonstrated conflicting findings in terms of the degeneration of the vestibular systems. In a patient with MIDD, the vestibular end-organs, including the utricle and semicircular canals, were well preserved [6], whereas two patients with MELAS showed degenerative changes in the vestibular end-organs and Scarpa's ganglions [29]. In one of these MELAS patients, there was a pathological collapse of the membranous wall of the saccule and significant hair cell loss in the saccular macula, utricular maculae, and the cristae of all three semicircular canals [29]. In another patient with MELAS, the hair cells in both cristae and maculae were reduced, and the numbers of Scarpa ganglion cells were reduced to approximately 70% of the mean counts of age-matched control samples [29].

It has been reported that the disease severity and the expression pattern of the impairment across organs and tissues may vary in mitochondrial diseases, depending on the heteroplasmy levels. In general, patients with a higher heteroplasmy level exhibit more severe phenotypes, although the correlation may be weak [26,36–38]. Since the heteroplasmy level in the inner ear cannot be assessed clinically, we adopted the heteroplasmy level in peripheral leucocytes and found that the heteroplasmy level correlated with the onset of hearing loss; hearing loss developed at a younger age in patients with higher heteroplasmy. Previous studies also demonstrated that hearing loss developed earlier in patients with higher heteroplasmy levels [7,11]. The onset of balance–gait disorder correlated weakly with heteroplasmy level but significantly with age-corrected heteroplasmy level. The heteroplasmy level and age-corrected heteroplasmy level did not correlate with the onset of diabetes mellitus. Iwasaki et al. (2011) reported no correlation between heteroplasmy and onset of balance or gait disorder in 13 unrelated patients with m.3243A>G mutation [18]. Other studies showed a negative correlation between the onset of diabetes and heteroplasmy [11,39]. These discrepancies may be due to the small sample sizes, different phenotypes, and different stages of disease among reports. For example, in the current study, only patients with complaints of hearing loss were enrolled; therefore, there were two patients who did not have diabetes mellitus at the time of initial examination. Such bias in patient selection may have resulted in different correlations between the heteroplasmy levels and the onset of diabetes mellitus or balance–gait disorder.

It is worthy to note that the heteroplasmy and age-corrected heteroplasmy levels did not correlate with the progression rate of hearing loss or the presence of a rapid decline in hearing in our cases. It is unclear whether such a trend is also observed in other organs or tissues. De Laat et al. [36] scored disease severity using the Newcastle Mitochondrial Disease Adult Scale (NMDAS), including SF-36 quality of life (QoL) scores, and measured heteroplasmy levels in urinary epithelial cells, leucocytes, and saliva in 151 carriers of m.3243A>G mutation of mtDNA; their results indicate a yearly increase in NMDAS score of 0.47 point in the total group and that heteroplasmy levels in both leucocytes and urinary epithelial cells were only weakly correlated with disease severity. They also observed that physical QoL declined with age and that the most important determinants of QoL decline were hearing loss, speech problems, exercise intolerance, gait instability, psychiatric problems, and gastrointestinal involvement.

No medication is known to prevent or slow the progression of hearing loss associated with m.3243A>G mutation of mtDNA. Since the hearing loss involves mainly the cochlea, hearing aids are effective until the hearing loss becomes severe to profound. When hearing aids become ineffective for oral communication, cochlear implantation, which directly stimulates the auditory nerve, is recommended [40]. In the present study, six patients received a cochlear implant at our department, and all patients except one who developed cerebral

stroke 5 months after the cochlear implant surgery achieved good speech perception. As mentioned above, temporal bone histopathological studies demonstrated that the spiral ganglion cells were relatively well preserved in a patient with MIDD and degenerated in patients with MELAS, whereas the stria vascularis was severely degenerated [26,28]. Since hearing thresholds mainly reflects the functions of the cochlear cells, such as the hair cells and stria vascularis, it is presumed that the retro-cochlear auditory pathways, including the spiral ganglion cells, are still relatively well preserved when patients become profoundly deaf, which explains the high efficacy of cochlear implants. However, during long-term follow-up in the patients who have cochlear implantation with mitochondrial gene mutation, some patients who initially showed good speech perception exhibited deterioration of speech perception [41]. Therefore, patients receiving cochlear implantation should be carefully monitored over the long term.

5. Conclusions

This study evaluating the hearing in 15 patients with m.3243A>G mutation in mtDNA in the long-term period demonstrated that hearing loss occurred from 10 to 56 years of age, that the age of onset of hearing loss was correlated with heteroplasmy and age-corrected heteroplasmy levels, that the hearing loss progressed gradually initially, and that the rapid decline in hearing loss to profound deafness occurred in approximately half of the patients during the observation period. The hearing level prior to the rapid decline ranged from 56 to 80 dB HL, and the progression rate of hearing loss prior to the rapid decline was not significantly different from that in the remaining patients, who did not experience a rapid decline in hearing. Heteroplasmy and age-corrected heteroplasmy levels did not correlate with the presence of rapid progression of hearing loss or with the progression rate of hearing loss. These results indicate that it is difficult to predict the rapid decline in hearing in patients with m.3243A>G mutation of mtDNA. Neither oral nor systemic steroid treatment was effective in improving hearing loss.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/life12040543/s1, Figure S1: Chronological progression of hearing level from the first visit, Figure S2: Relationship between patients' age and progression of their hearing loss, Figure S3: Chronological change in hearing level from the first visit in patients who showed rapid decline in hearing.

Author Contributions: Conceptualization, A.S., A.K. and T.Y.; methodology, A.S., A.K. and T.Y.; formal analysis, A.S., A.K. and T.Y.; investigation, A.S., A.K., H.K., T.U., S.I., C.F., M.K. and T.Y.; writing—original draft preparation, A.S., A.K. and T.Y.; writing—review and editing, A.S., A.K., H.K., C.F. and T.Y.; supervision, T.Y.; project administration, T.Y. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Regional Ethical Standards Committee of the Faculty of Medicine at the University of Tokyo (Application Number 2487).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; in the decision to publish the results.

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Communication

Cochlear Implantation in Patients with Mitochondrial Gene Mutation: Decline in Speech Perception in Retrospective Long-Term Follow-Up Study

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Abstract: Clinical evidence of the effectiveness of cochlear implantation for hearing loss with mitochondrial DNA mutation is limited. Most reports have only described short-term postoperative speech perception, which may not reflect the limitations of cochlear implantation caused by progressive retrocochlear dysfunction. The present study aimed to investigate long-term speech perception after cochlear implantation in patients with severe to profound hearing loss associated with mitochondrial DNA mutation. A retrospective chart review was performed on patients with mitochondrial DNA mutation who had undergone cochlear implantation at the Department of Otolaryngology and Head and Neck Surgery at the University of Tokyo Hospital. We extracted data on causative mutations, clinical types, clinical course, perioperative complications, and short-term and long-term postoperative speech perception. Nine patients with mitochondrial DNA mutation underwent cochlear implantation. The mean observation period was 5.5 ± 4.2 years (range, 1–13 years), and seven patients were followed for more than 3 years. Two of the seven patients who initially showed good speech perception exhibited deterioration during long-term follow-up. The absence of an acute progression of cognitive decline in patients, showing a gradual decrease in speech perception, suggests that the deterioration of speech perception was caused by progressive retrocochlear degeneration. Although most patients with mitochondrial DNA mutation maintained good speech perception for more than 3 years after cochlear implantation, retrocochlear degeneration could cause the deterioration of speech perception during long-term follow-up.

Keywords: cochlear implantation; retrocochlear dysfunction; mitochondrial gene mutations

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1. Introduction

Mitochondria play an important role in intracellular adenosine triphosphate production by oxidative phosphorylation, an essential energy source in nucleated cells. Mutations in mitochondrial DNA (mtDNA) cause dysfunction, especially in tissues with high metabolic demands. In patients with mtDNA mutations, organs that rely on aerobic energy production, such as the visual pathway, heart, central nervous system, and skeletal muscle, are primarily affected. The auditory pathway, including the cochlea, also has large energy demand; therefore, the auditory pathway is an organ that can be profoundly affected by mitochondrial disorders [1–3].

More than half of the patients with mtDNA mutations are affected by a hearing impairment at some time during the disease course [4–6]. Pathological mutations of the mtDNA have been commonly found at the transfer RNAs (tRNAs). To date, more than 90 point mutations in 21 of the 22 mitochondrial tRNA genes have been reported [7,8]. Most of these mutations result in a decreased rate of mitochondrial protein synthesis, causing a deficiency in the energy metabolism of the cell [9]. Approximately 50 mutations of the tRNA genes have been associated with deafness [10]. Associated features of hearing loss include encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS syndrome), diabetes (maternally inherited diabetes and deafness, or MIDD, syndrome), ophthalmoplegia (chronic progressive external ophthalmoplegia, or CPEO, syndrome), cardiac conduction abnormalities with retinopathy and ophthalmoplegia (Kearns–Sayre syndrome), myoclonus epilepsy (myoclonus epilepsy associated with ragged-red fiber, or MERRF, syndrome), ptosis, ophthalmoplegia, gastrointestinal dysmotility, cachexia, peripheral neuropathy, and leukoencephalopathy (mitochondrial neurogastrointestinal encephalopathy, or MNGIE, syndrome). Hearing loss is usually gradual at onset, initially occurs at high frequencies, is predominantly bilaterally symmetrical, and progresses to profound. Hearing loss in patients with mitochondrial diseases is mainly attributed to cochlear dysfunction [11–15], but mitochondrial disorders can also affect the central nervous system, including the central auditory pathway, and can cause psychomotor regression [4,16–19]. Roesch et al. [20] conducted a systematic review of knowledge on hearing loss in genetically proven mitochondrial disease in children. A total of 75 patients from 23 studies were included in the analysis. Retrocochlear hearing loss was found more often (33 out of 75 patients) than expected. Affected genes included OPA1 in 14 patients, FDXR in seven patients, and MT-TL1 in six patients. The clinical courses of these patients, including the age of onset and disease severity, showed diverse characteristics. Takahashi et al. [21] reported histopathological examinations of human temporal bones in MELAS patients and found severe degeneration of the stria vascularis and the spiral ganglion cells. There was severe atrophy of the stria vascularis in all turns of the cochlea, and the remaining stria cells showed vacuole formation and the presence of small, dark-staining, round, and ovoid cells. Both the outer and inner hair cells were generally present, with scattered losses in the lower basal turns. In addition to these findings, the total number of spiral ganglion cells was reduced when compared with the mean values of normal newborn and age-matched control samples, representing a mild neuronal loss. Many spiral ganglion cells showed varying degrees of degenerative change, as evidenced by faint staining of the cytoplasm, loss of cell membrane outline, and loss of nuclear definition. Other histopathological examinations of the human temporal bone also demonstrated that mtDNA A3243G mutation can involve not only the stria vascularis and hair cells but also the spiral ganglion cells [22,23].

A defect in the inner hair cells, the auditory nerve, the connection between them, or the connection between the nerve and brain can lead to auditory neuropathy spectrum disorder (ANSD). ANSD has been reported to be associated with head injury; infections due to various viruses such as measles, mumps, and cytomegalovirus; and high fever and is also caused by specific gene mutations, such as OTOF. ANSD is characteristic of relatively mild hearing impairment with abnormal ABR response and poor speech recognition score, while distortion product otoacoustic emission (DPOAE) is normal [24,25]. In a report from Leruez et al. [26], 8 out of 19 patients with OPA1 gene mutation were reported to have suspected ANSD. Sakai et al. [27] reported a patient with normal DPOAE who had fluctuation of hearing threshold measured by ABR; because the peak latency of wave I and wave V and the intervals of waves I–V were markedly delayed, the existence of a retrocochlear problem was speculated to be a cause of hearing loss.

Cochlear implantation (CI) for patients with severe to profound hearing loss associated with mtDNA mutations has been reported [19,21–23,28–35]. Howes et al. [36] reported a case of MIDD with a speech score of 67% at one-month follow-up. Yasumura et al. [37] reported a case of MELAS with a speech score of 72% at 3-month follow-up. Li et al. [34] reported a case of MNGIE with a speech score of 56% at 3-month follow-up. All of these

reports emphasized that CI is generally effective for patients with mtDNA mutations, but most of them only described speech perception in the short-term postoperative period. Therefore, it is unclear whether the effectiveness of CI is limited by the progression of retrocochlear dysfunction and/or cognitive decline associated with mitochondrial disorder. In fact, a patient has been reported to show poor postoperative speech perception associated with cognitive problems in relatively long-term follow-up [38].

In the present study, we investigated not only short-term but also long-term speech perception after CI in patients with profound hearing loss associated with mtDNA mutation.

2. Materials and Methods

A retrospective chart review was performed on patients who had undergone CI at the Department of Otolaryngology and Head and Neck Surgery at the University of Tokyo Hospital from 1991 to 2019. Nine patients were diagnosed with mtDNA mutations via genetic testing, and the additional information extracted included the causative mutations, clinical types, clinical course, perioperative complications, and postoperative speech perception. The Fukuda version of the monosyllabic speech perception test was used to evaluate speech perception before and after CI. Speech performance in noise was evaluated in four patients, including two patients examined twice, using a CI-2004 Japanese open-set sentence test. Tests were performed in quiet, SN20 and SN10. A DPOAE test and a promontory stimulation test were performed to differentiate between retrocochlear and cochlear hearing loss. Cases with obvious decline in attention, executive function, learning/memory, language, perceptual/motor functions, and social cognitive functions during the examination or as reported by family members were considered to have cognitive deterioration. The present study was approved by the Regional Ethical Standards Committee of the Faculty of Medicine at the University of Tokyo (application number 2487) and was conducted in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from the patients for publication of this study.

3. Results

3.1. Patient Characteristics

The characteristics of the nine patients with mtDNA mutations who underwent CI are shown in Table 1. The mean age at CI was 45.0 ± 11.5 years (range, 22–64 years), and the mean observation period was 5.5 ± 4.2 years (range, 1–13 years). A3243G mutation was identified in seven patients and RRM2B mutation and A8296G mutation were each identified in one patient. Of the seven patients with A3243G mutation, six patients were diagnosed with MIDD, and one was diagnosed with MELAS. A patient with the RRM2B mutation was diagnosed with CPEO, and a patient with the A8296G mutation only had hearing loss. Among the subjects, there were no suspicious findings of cognitive decline preoperatively. No patients showed any response in DPOAE tests, indicating that hearing loss involved the cochlea. All patients except one (patient 7) showed good response in promontory stimulation tests, which indicates that the retrocochlear auditory pathway was markedly involved in patient 7 but not in others.

Table 1. Summary of patients.

Patient	Disease	Causative Mutations	Age of Onset of Hearing Loss (years)	Age of Becoming Deaf (years)	Age at CI (years)	Observation Period (years)	Associated Symptoms
1	MIDD	A3243G	30	53	53	3.4	diabetes
2	MIDD	A3243G	32	46	46	13	diabetes
3	MIDD	A3243G	38	44	44	12.2	diabetes
4	MIDD	A3243G	27	64	64	1.2	diabetes
5	MIDD	A3243G	10	50	51	6.2	diabetes
6	MIDD	A3243G	14	36	37	3.1	diabetes
7	MELAS	A3243G	10	44	46	4.0	myopathy, lactic acidosis, stroke-like episode
8	–	A8296G	7	21	22	4.5	-
9	CPEO	RRM2Bs	5	43	43	2.2	mild external ophthalmoplegia

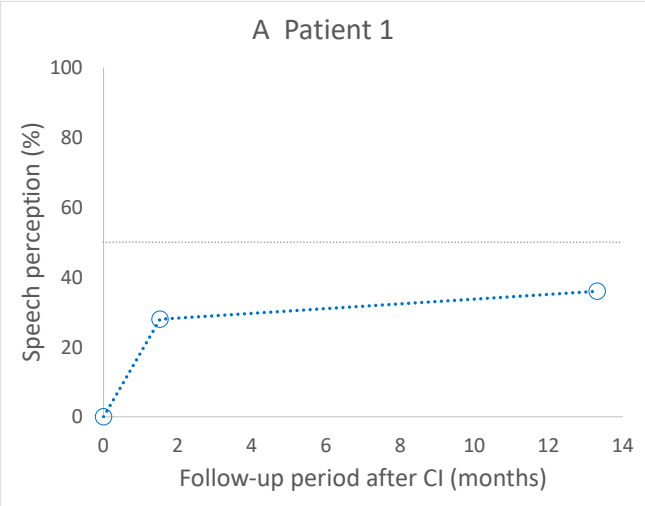
Abbreviations: MIDD, maternally inherited diabetes with deafness; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes; CPEO, chronic progressive external ophthalmoplegia; CI, cochlear implantation.

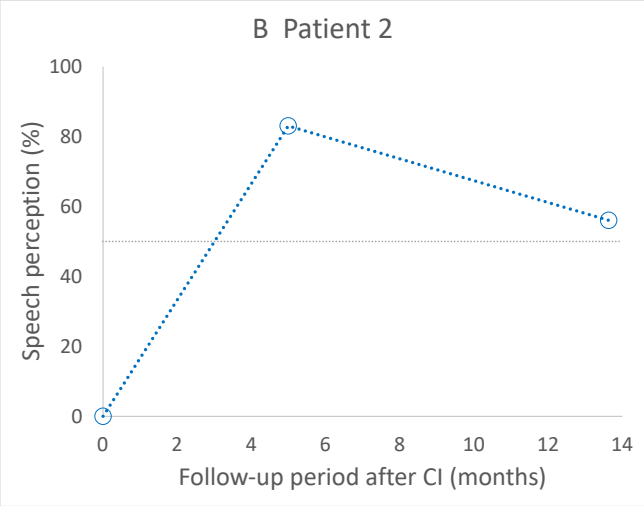
3.2. Surgical Findings

No complications were observed during the surgery in any patient. A CI24M (Cochlear®, Lane Cove, Australia) electrode was used in patients 1, 2, and 3; a CI24RE (Cochlear®) electrode in patients 4, 5, 6, 7, and 9; and a CI422 (Cochlear®) electrode in patient 8. As there were no malformed cochlear cases in this series, we chose the latest electrode at surgery. All patients received CI only in the unilateral ear. Full insertion of CI electrodes was achieved in all patients. Electrically evoked compound action potentials were detected in all electrodes in all patients.

3.3. Postoperative Speech Perception

Postoperative speech perception results within 14 months after CI are shown in Figure 1. Seven patients achieved scores of ≥50% in the Fukuda version of the monosyllabic speech perception test after CI, whereas two patients achieved scores of <50% (patients 1 and 3).





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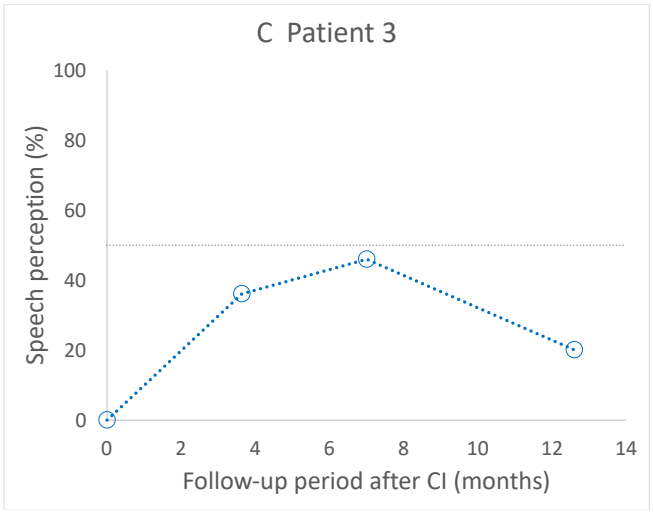

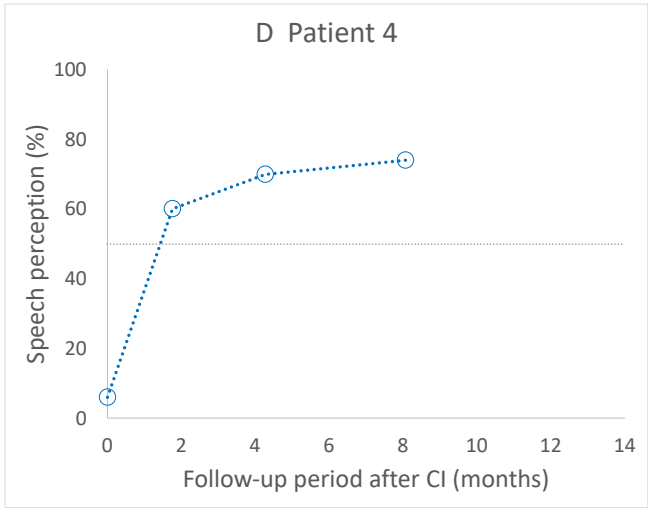

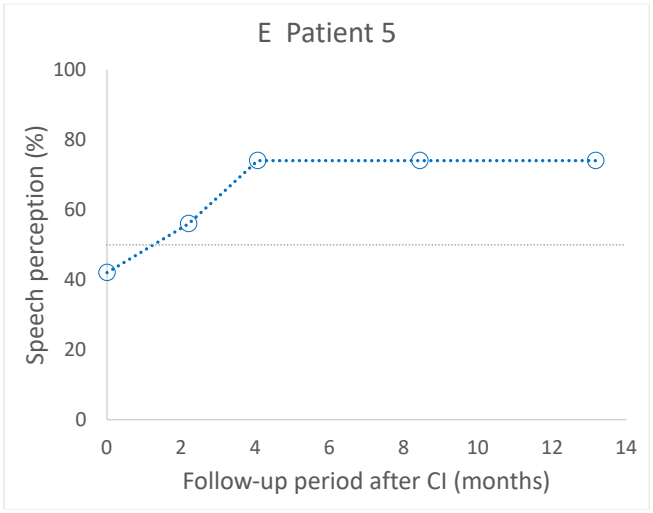

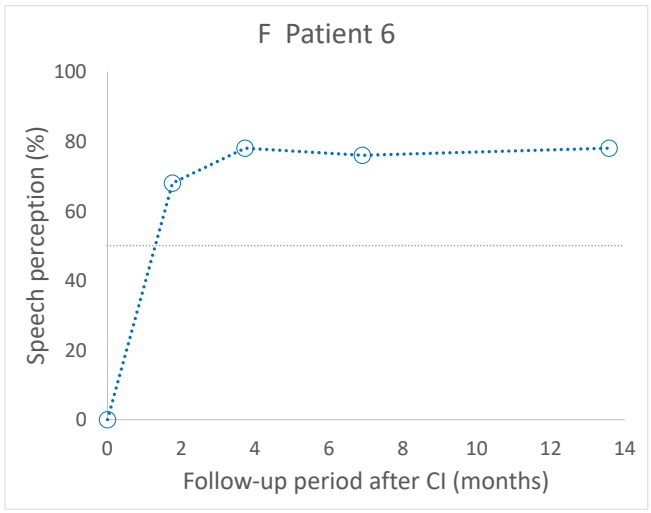

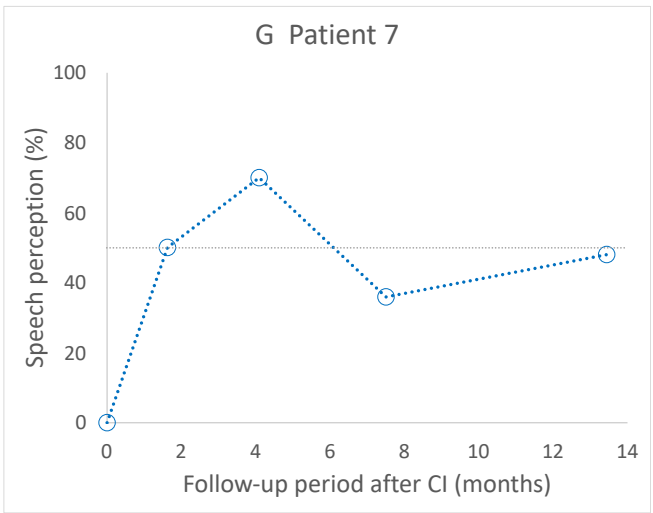

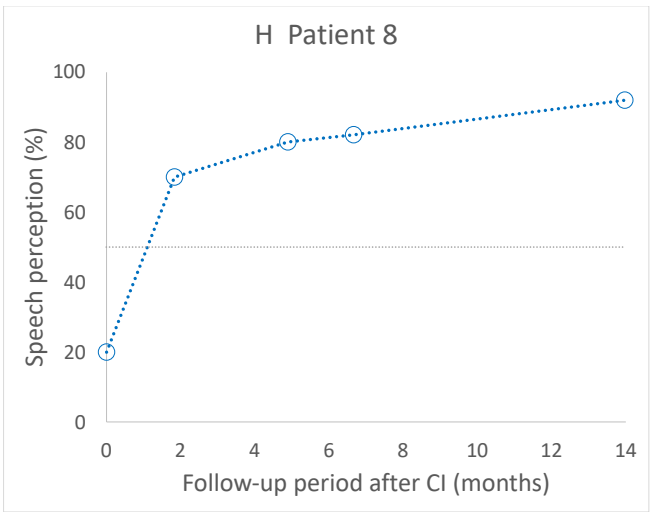

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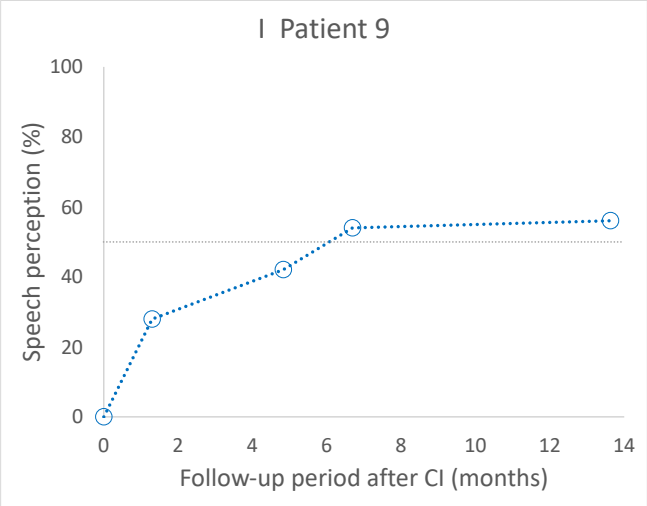

Figure 1. Short-term results of postoperative speech perception. Seven patients (**B**,**D**–**I**) achieved scores ≥ 50% after CI, and two patients (**A**,**C**) achieved poorer outcomes. CI, cochlear implantation.

The results of long-term postoperative speech perception are shown in Figure 2. Of the seven patients who were followed for more than 3 years, three patients (patients 2, 3, and 5) were followed for more than 5 years. Three patients (patients 2, 3, and 7) showed a decrease in postoperative speech perception of 20% or more. Patient 2 had no identifiable reasons for an acute deterioration in the first year and a gradual deterioration during the long-term follow-up. There was no sign of device failure, such as increasing impedances, an increase in clinical threshold level, or a reduced number of available electrodes in the course of deterioration of speech perception. In patient 3, a temporal shift in speech perception improved after mapping modification, and thereafter, no changes were observed during the long-term follow-up period. In patient 7, an acute deterioration in the first year was attributed to high-order brain dysfunction caused by cerebral infarction, but this episode did not cause the limited usage of the implant or make it difficult to conduct a speech perception test. After this episode, she showed a progressive decline in speech perception, despite the absence of an additional central episode or cognitive decline. There was no sign of device failure, such as increasing impedances, increases in threshold level by NRT, increases in clinical threshold level, or a reduced number of available electrodes in the course of deterioration of speech perception.

The results of sentence recognition tests in noise in four patients are shown in Table 2. Noise significantly influenced speech perception in one patient (patient 4), showing a poor score even in quiet conditions; this patient showed a progressive decline in the monosyllabic speech perception test. The other three patients maintained good scores under noise exposure, and two of them, who were examined twice using a sentence recognition test in noise, showed stable performance for more than three years; these patients also showed stable performance in long-term monosyllabic speech perception tests.

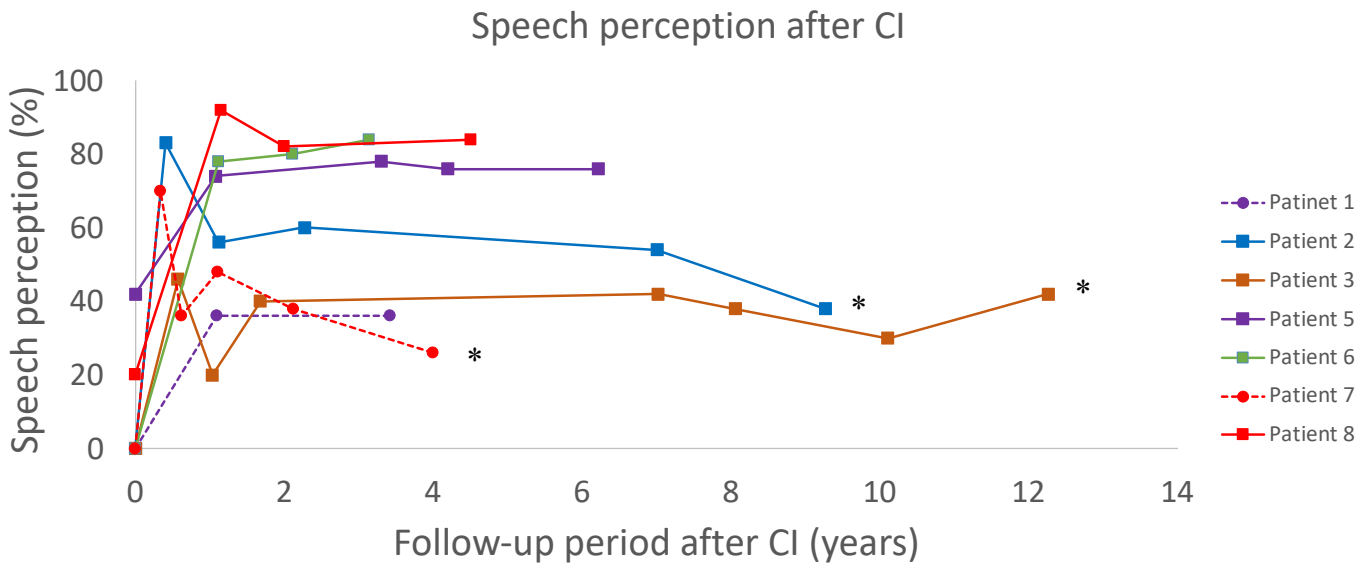

Figure 2. Long-term results of postoperative speech perception. Postoperative speech perception for seven patients. * Three patients with speech perception reduced by 20% or more.

Table 2. Results of speech in noise test.

Patient	Tested Year (Years after CI)	In Quiet (%)	S/N20 (%)	S/N10 (%)
4	5.2	45	20	-
5	1.3	95	95	58
	3.9	100	95	78
6	1.5	98	97	73
8	1.5	98	100	57
	3	100	92	-

A CI 2004 speech test was conducted in four patients at some point after CI. CI, cochlear implantation.

4. Discussion

In the present study, we investigated the short-term and long-term postoperative speech perception in nine patients who underwent CI for profound hearing loss with mtDNA mutation. Seven patients exhibited a good score of ≥50% in the Fukuda version of the monosyllabic speech perception test during the first postoperative year, but two of the seven patients showed deterioration during the long-term follow-up period. The short-term results in the current study agree with those in previous reports. Sinnathuray et al. [6] compared the results of postoperative speech perception in 12 patients with mtDNA mutations from 1997 to 2002 and reported good results irrespective of disease type, severity, and duration of hearing loss. Nawal et al. [39] conducted a systematic review of cochlear implantation outcomes in patients with mitochondrial hearing loss. In that study, 13 patients from 11 studies performed speech perception tests, and 10 out of 11 patients scored more than 50% in either speech, word, or phoneme recognition tests.

Deafness associated with mtDNA abnormalities is mainly attributed to dysfunction of the inner ear [11–15], but the retrocochlear auditory pathway may also be involved [4,16,17,21–23]. Short-term improvements in speech perception after CI may wane during long-term follow-up due to the degeneration of the spiral ganglion cells or cognitive decline due to progressive mitochondrial disorder. In a recently reported retrospective case series of five patients with mitochondrial diseases, including MELAS and MIDD, speech perception was preserved during the long-term follow-up period in four patients, but one patient could only use implants for several hours per day and could not conduct the speech perception test within 2 years of surgery [38]. In that report, the authors

speculated that cognitive decline from the disease made the patient unable to recognize the importance of using the implant for the establishment of speech perception.

In the current study, two patients who initially achieved good speech perception exhibited a decrease in speech perception during the long-term follow-up period. In patient 2, neither cognitive decline nor deterioration of the device itself, such as a decrease in the number of available electrodes or an increase in the impedance of electrodes, were observed; therefore, progressive retrocochlear dysfunction was considered as the cause of the deterioration in speech perception. In another patient (patient 7), the initial decline in speech perception was associated with cerebral infarction, but the absence of additional central episodes, cognitive decline, or deterioration of the device itself thereafter suggests that the decline in speech perception during the long-term follow-up after cerebral infarction may be associated with progressive retrocochlear impairment associated with mtDNA mutation.

Previous studies [22–37,40,41] and short-term observations in the present study indicate that patients with mtDNA mutations are good candidates for CI. Notably, however, the long-term observations in the present study also suggest that retrocochlear dysfunction may be responsible for the long-term deterioration of speech perception after CI in patients with mtDNA mutations. Several reports [42–44] have investigated long-term speech performance in patients receiving CI and have observed no decline in speech perception performance. For example, Hilly et al. [42] examined 87 cochlear implant recipients, including 22 patients over 70 years of age, with a mean follow-up of 6.8 years, and found that most patients had a stable outcome during the follow-up period. Even in patients who are older, 13.6 percent improved and none had a reduction in score of more than 20 percent. Dillon et al. [43] followed 14 cochlear implant recipients aged 65 years and older for at least 10 years and found that consonant–nucleus–consonant word scores were stable between 6 months and 1 year of listening experience, improved significantly between 1 year and 5 years, and were stable between 5 years and 10 years. Hearing in Noise Test sentence scores in quiet and in noise showed a similar pattern, with stability in performance between the 6-month to 1-year and 5-year to 10-year follow-up intervals, and significantly improved performance between the 1-year and 5-year follow-up intervals. Therefore, diagnosis of mitochondrial diseases will have an impact on long-term performance of CI as well as future progression of hearing loss. Although CI has the potential to improve the quality of life in these patients, surgeons need to provide information about the possibility of gradual deterioration of speech perception in the long term after CI, so that patients and their families can prepare their future living environments and support. At our institution, we evaluate for mitochondrial genetic abnormalities at the time of initial consultation in patients with symptoms and signs suggestive of maternal inheritance.

Because no objective assessment data were available to confirm the progression of retrocochlear dysfunction, there were no clear predictors of the deterioration of speech perception during the long-term follow-up period. Preoperative diagnosis of retrocochlear involvement may have some impact on long-term performance of CI. All patients in our case series showed an absence of DPOAE response, indicating cochlear involvement. Absent or poor response in promontory stimulation tests indicates retrocochlear dysfunction. In the present study, two patients showed deterioration of speech performance during follow-up; one of them (patient 7) showed poor response in the promontory stimulation test, but the other (patient 2) showed good response. Therefore, it is unclear if preoperative retrocochlear involvement can predict the decline in speech perception in the long-term period. Breneman et al. [45] reported a long-term outcome of cochlear implantation in patients with auditory neuropathy spectrum disorder (ANSD). In that study, 35 patients with a follow-up period of more than six years on average showed as good a response as children with non-ANSD SNHL, which suggests that diagnosis of retrocochlear disease is not sufficient to predict the long-term benefit of CI. Superficial hemosiderosis is also known to present retrocochlear deafness. In a systematic review by Chaudhry et al. [46], 31 out of 44 patients showed improved hearing outcomes following CI, and 22 implants had sustained benefit at the last follow-up. They concluded that longevity of benefit was diffi-

cult to predict because of the progressive nature of the disease and a lack of preoperative prognosticators. Pijl et al. [41] used electrically evoked auditory brainstem response (EABR) and middle latency response (MLR) data derived from two patients with Kearns–Sayre syndrome and found that EABR and MLR were useful for distinguishing between cochlear and retrocochlear hearing loss and for predicting outcomes after CI. Rosenthal et al. [40] reported EABR and MLR data derived from a patient with MELAS syndrome who had significant central nervous system deficits, and proposed prioritizing MLR testing rather than EABR to evaluate the integrity of the auditory pathway. Introduction of these examinations may be useful for the prediction of future performance.

There have been several reports that older adult cochlear implant users have poorer performance of speech in noise compared to younger adults. This may be due to the fact that listening in noise is more susceptible to retrocochlear auditory pathway damage [47,48]. Although the present study could not provide sufficient data, it may be possible to predict the deterioration of speech performance with progression of retrocochlear dysfunction by repeated evaluation under noise conditions.

Generally, central nervous system symptoms, such as stroke-like episodes in MELAS patients, progress slowly [49,50]. Therefore, retrocochlear dysfunction after CI is also likely to progress slowly. Long-term observation may reveal deterioration of speech perception in our patients followed for less than 5 years.

It should be noted that heterogeneity of the presented samples may affect the interpretation of the results because of the relatively small number of cases. Although the time from deafness to surgery was around one year in most cases, there was diversity in the onset of hearing loss and the age of surgery. Heteroplasmy is also known to affect disease severity and the expression pattern of the impairment across organs and tissues [51,52], but was not analyzed in this study.

5. Conclusions

We retrospectively reviewed short-term and long-term speech perception after CI in nine patients with deafness associated with mtDNA mutations. Two of the seven patients who initially achieved good speech perception scores exhibited a deterioration in speech perception during the long-term follow-up. The absence of acute progression of cognitive decline in conjunction with the gradual decline in speech perception suggests that retrocochlear dysfunction associated with mitochondrial disorder could be responsible for the deterioration of speech perception.

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Original article

Meaningful word acquisition is associated with walking ability over 10 years in Rett syndrome

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Abstract

Purpose: To investigate walking ability in Japanese patients with Rett syndrome (RTT).

Methods: Walking ability was assessed in 100 female Japanese patients with RTT using univariate and multivariate analysis in all age groups, and in patients over 10 years of age. We analyzed walking ability and confounding factors including prenatal-perinatal histories, developmental milestones, somatic and head growth, anthropometric data, body mass index, age of loss of purposeful hand use, age at onset of stereotypic hand movement, history of autistic behavior, age at regression, presence or absence of seizures, and the results of *MECP2* genetic examination from the Japanese Rett syndrome database.

Results: Univariate analysis revealed that acquisition of walking in all age groups was significantly correlated with the acquisition of meaningful words, microcephaly, and crawling ($P < 0.0001$, $P = 0.005$, $P < 0.0001$, respectively). Univariate analysis revealed that walking ability over 10 years of age was significantly correlated with acquisition of meaningful words, microcephaly, and body mass index ($P < 0{,}0001$, $P = 0.005$, $P = 0.0018$, respectively). *MECP2* mutations R306C, R133C, and R294X were significantly associated with different acquisition of crawling ($P = 0.004$) and walking ($P = 0.01$). Multivariate analysis revealed that only acquisition of meaningful words was significantly correlated with walking ability over 10 years of age. This trend excluded the genetic effects of R306C, R133C, and R294X.

Conclusions: Meaningful word acquisition was robustly associated with walking ability over 10 years. Prognosis of walking ability may be predicted by the acquisition of meaningful words. This information is potentially useful for early intervention and the planning of comprehensive treatment for young children with RTT.

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1. Introduction

Rett syndrome (RTT) (OMIM #312750) is a neurodevelopmental disorder primarily affecting females. Most cases of RTT are caused by de novo mutations in the gene encoding methyl-CpG binding protein 2 (MECP2) [1]. Approximately 90%–95% of typical RTT cases exhibit loss-of-function mutations in the *MECP2* gene of the X-chromosome [2].

Clinical manifestations include microcephaly, loss of psychomotor abilities, intellectual disability (ID), autistic behaviors, and hand stereotypies. Recent large cross-sectional studies revealed substantial clinical variability in *MECP2* mutations [2]. It has been reported that girls and women with the mutations p.Arg270* (R270X) or p.Arg255* (R255X) present with more severe motor disability, whereas those with mutations p.Arg306Cys*, p.Arg133Cys*, and p.Arg294* (R306C, R133C, R294X, respectively), and C-terminal deletions exhibit a milder phenotype, and, in most cases, acquire the ability to walk [3–7]. However, it is difficult to estimate genotype-phenotype correlations because of the role of X inactivation. In the present study, we utilized the Japanese Rett syndrome database (JRSD), which was established in October 2012 and contains clinical data from over 102 RTT patients. Walking ability is important for family counselling and planning for the provision of care for young children with RTT. In the current study, we investigated factors related to ambulation ability in young children over 10 years of age with or without genetic effects, based on the JRSD.

2. Patients and methods

2.1. Japanese Rett syndrome database and subjects

The JRSD is operated by management officers consisting of child neurologists and RTT family associations, and is supported by the Japanese government. The registration document is completed with a combination of parents' questionnaire responses and doctor's examination data after children's diagnosis with RTT with clinical criteria and/or genetic abnormality [2]. A total of 102 female patients with RTT were registered from October 2012 to December 2015. For the current study, we obtained informed consent from all RTT parents and ethical approval from each institution. The JRSD registration document contains prenatal/perinatal history, developmental milestones, somatic and head growth, anthropometric data, growth status (body mass index [BMI]), age at regression, age at development of stereotypic hand movement, loss of purposeful hand function, history and age of onset of autistic behavior, eating and swallowing ability, vocalization/verbalization, periodic breathing, hand and foot temperature, dystonia, tremor, seizures, scoliosis, muscle tonus, dental and oral problems, and genetic examinations.

2.2. Gene testing

Total genomic DNA was prepared from peripheral blood leukocytes according to standard procedures. Participants in this study underwent complete *MECP2* mutation analysis, including exon 1 and evaluation for large DNA rearrangement, by Southern blotting or by multiple ligation-dependent probe amplification (MLPA) analysis. Whole exome sequencing (WES) was performed in some patients, as previously described [8].

2.3. Sentinel surveillance and statistical analyses

The Kaplan–Meier method was used to estimate survival curves and the log-rank test was used to compare estimated survival curves. In addition, univariate and multivariate logistic regression models were employed to test the relationships between walking ability over 10 years of age and other factors. All variables were visually inspected to assess their distribution. When variables were judged to be skewed and the normality assumption was not tenable, non-parametric tests, Spearman's rank correlations and Mann-Whitney U-tests were employed. χ^2 test and Fisher's exact test were used to examine the relationships between categorical variables. P-values less than 0.05 were considered to indicate significant differences. JMP Pro 13 (SAS institute, Cary, NC, USA) was used to perform all data analyses. The research protocol was approved by the Ethics Committees of National Center of Neurology and Psychiatry, and the Ethics Committees of each participating institution.

3. Results

Two patients were excluded from the study because of incomplete data for RTT diagnosis, according to recent clinical diagnostic criteria [2]. We analyzed registered data from 86 typical and 14 atypical RTT patients. All patients were female, and ages ranged from 1 year to 43 years of age (mean ± SD; 14.5 ± 11.2 years of age;

Table 1
Clinical distribution in Japanese Rett Syndrome Database.

Symptoms	Age at registration					
	1–5 yrs	6–10yrs	11–15yrs	16–20yrs	>21yrs	Total
Number of patients	19	25	18	6	32	100
Main criteria						
Partial/complete loss of acquired purposeful hand skills	19 (1 0 0)	23 (92.0)	14 (77.8)	5 (83.3)	25 (78.1)	86 (86)
Partial/complete loss of acquired spoken language	10 (52.6)	13 (52.0)	12 (66.7)	4 (66.7)	21 (68.8)	60 (60)
Gait abnormalities	17 (89.4)	22 (88.0)	17 (94.4)	6 (100)	30 (93.8)	92 (92)
Stereotypic hand movements	19 (100)	24 (96.0)	18 (100)	6 (100)	32 (100)	99 (99)
Supportive criteria						
Breathing disturbances when awake	11 (57.8)	17 (68.0)	13 (72.2)	6 (100)	18 (56.3)	65 (65)
Bruxism when awake	14 (73.6)	20 (80.0)	13 (72.2)	5 (83.3)	18 (56.3)	70 (70)
Impaired sleep pattern	11 (57.8)	14 (56.0)	8 (44.4)	6 (100)	22 (68.8)	61 (61)
Abnormal muscle tone	19 (100)	19 (76.0)	15 (83.3)	6 (100)	29 (90.6)	88 (88)
Peripheral vasomotor disturbances	18 (94.7)	24 (96.0)	18 (100)	6 (100)	28 (87.5)	94 (94)
Scoliosis/kyphosis	3 (15.7)	12 (48.0)	14 (77.8)	6 (100)	29 (90.6)	64 (64)
Inappropriate laughing/screaming spells	18 (94.7)	20 (80.0)	15 (83.3)	6 (100)	20 (62.5)	79 (79)
Autistic behavior with intense eye communication	16 (84.2)	16 (64.0)	13 (72.2)	6 (100)	24 (75.0)	75 (75)
Epilepsy	7 (36.8)	16 (64.0)	13 (83.3)	6 (100)	26 (81.3)	68 (68)

N = number of patients; yrs = years.

Table 2
Distribution of *MECP2* mutations in Japanese Rett Syndrome Database.

	Number of patients per total
MECP2 mutation examination	92/102 (90.2%)
MECP2 mutations, identified	88/92 (95%)
Typical	86/102 (84%)
Genotype	N(%)
R168X	11 (11.8)
T158M	8 (8.6)
R255X	8 (6.4)
R270X	5 (5.4)
R294X	5 (5.4)
R133C	5 (5.4)
R306C	4 (4.3)
R306W	3 (3.2)

median; 11.4 years of age). Table 1 shows the age distribution and frequency of symptoms and signs. Of 100 RTT patients, 92 (92%) underwent genetic examination by Southern blotting, MLPA, or WES.

Of these patients, 88 (95%) exhibited various *MECP2* mutations (Table 2). *MECP2* mutations included R168X (11 patients) (12.5%), T158M (eight patients) (9%) and R255X (six patients) (6.8%) (Table 2). All patients developed head control (median; 4 months of age). Ninety-seven patients acquired the ability to roll over (median; 6 months), while three were never able to roll over (3%). Seventy-nine patients were able to sit unassisted (median; 8 months) (79%). Fifty-six patients acquired the ability to crawl (median; 11 months) (56%). Forty-nine patients did not acquire the ability to walk without support (median; 18 months) (49%). The age of acquisition of walking ranged from 11 months to 72 months (median: 18 months). Forty patients did not develop the ability to produce meaningful words (40%). Among the other patients, the age of first meaningful words ranged from 10 months to 72 months of age. Fifty-eight patients exhibited a severely small head circumference (56%).

3.1. Correlation of acquisition of walking ability, meaningful words and microcephaly

We hypothesized that walking ability over 10 years of age may be related to a range of factors highlighted in previous studies, as follows: acquisition of meaningful words, presence or absence of microcephaly, dystonia, abnormal muscle tone, breathing abnormalities, age of onset of stereotypic hand movement, age of regression of hand function, and presence or absence of scoliosis [9–12].

First, we analyzed all data related to motor function, then examined acquisition of walking ability in all age groups. The Kaplan–Meier method was used to assess the relationships between acquisition of head control, sitting alone, crawling, meaningful words, microcephaly and acquisition of walking. Fig. 1 shows the estimated mean proportion of patients who acquired walking at all ages, for 100 patients. The proportion of patients who acquired the ability to walk increased until 100 months, then tended to flatten. Patients who acquired meaningful words showed a significantly greater rate of acquiring walking than those who did not acquire meaningful words, for all age groups ($P < 0.001$). Patients with absence of microcephaly exhibited a significantly greater rate of acquiring of walking than those with microcephaly ($P = 0.005$).

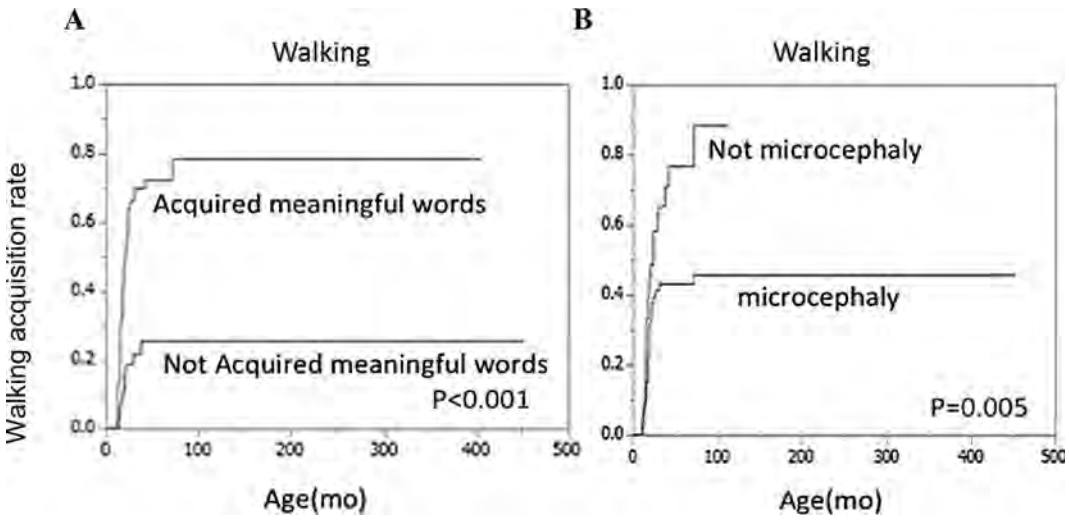

Fig. 1. Relationship between walking ability, acquisition of meaningful speech and microcephaly Walking ability was significantly different between patients who acquired meaningful words and those who did not acquire meaningful words, $P < 0.001$ (A). Walking ability was significantly different between patients with and without microcephaly, $P = 0.005$ (B), Kaplan–Meier method.

3.2. Acquisition of walking and effect of MECP2 mutation type (R306C, R133C, R294X)

Genotype *MECP2* mutation severity was categorized into mild (R306C, R133C, R294X) and other, based on previous reports [13]. To assess acquisition of walking, we also excluded R306C, R133C, R294X gene mutations, because an increasing number of patients with RTT did not undergo MECP2 gene testing in recent years. Fig. 2 shows an analysis of the acquisition of crawling (Fig. 2A) and walking (Fig. 2B) in relation to gene mutations, including R306C, R133C, R294X and other mutations. The rate of acquisition of crawling increased with age, until 30 months (Fig. 2A), and the number of patients that acquired walking increased until 40 months of age, then tended to flatten (Fig. 2B). Patients with mild phenotype mutations (R306C, R133C, R294X) exhibited significantly higher rates of acquiring crawling and walking ($P = 0.004$, $P = 0.01$, respectively).

3.3. Analysis of walking ability over 10 years of age

Age distribution was observed and analyzed in two age categories: over 10 years old, and all ages [12,13]. According to previous studies of motor symptoms

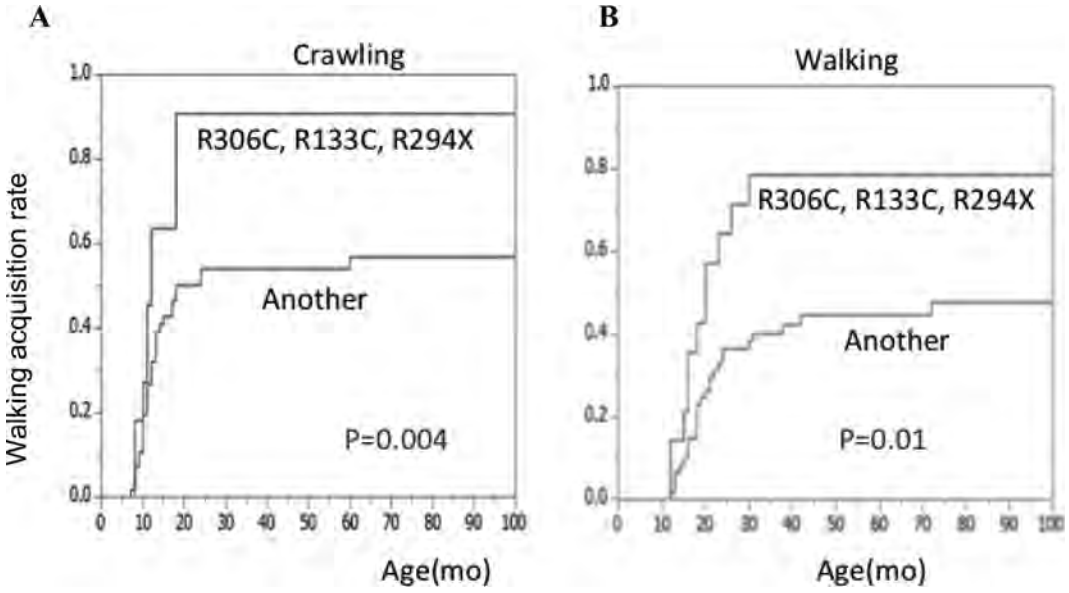

Fig. 2. Crawling and walking ability were significantly different between patients with *MECP2* mutations of R306C, R133C, R294X and other mutations Crawling ability and walking ability were significantly different between patients with *MECP2* mutations of R306C, R133C, R294X and other mutations. $P = 0.004$ (A), and $P = 0.01$ (B), respectively, Kaplan–Meier method.

including walking, we divided patients into four groups, as follows: those currently able to walk, those who were previously able to walk then lost walking ability, those unable to walk at over 10 years of age, and those with unknown walking status (Fig. 3). Of 56 patients over 10 years old, 31 were currently able to walk, 17 patients never learned to walk, six patients had lost the ability to walk, and two patients had unknown walking status. We divided participants into two groups depending on walking prognosis, as follows: participants who were still walking after 10 years of age, and a group of participants who were able to walk previously then lost the ability to walk, or who had never walked (Figs. 3, 4). The Kaplan–Meier method was used to assess the relationships between acquisition of crawling, acquisition of meaningful words, and walking ability over 10 years of age. The results shown in the figures indicate that walking ability was related to the acquisition of crawling (Fig. 4A), and the acquisition of meaningful words (Fig. 4B) over 10 years of age. As shown in Fig. 4, the proportion of patients with the ability to walk over 10 years of age was significantly related to the acquisition of crawling, and the acquisition of meaningful words ($P < 0.001$, $P < 0.001$, respectively).

Univariate analysis and multivariate analysis were used to assess the relationships between walking ability and early clinical signs. Univariate analysis revealed that walking ability was significantly correlated with meaningful word acquisition, microcephaly and BMI ($P < 0.0001$, $P = 0.005$, $P = 0.0018$, respectively). Lower BMI was negatively correlated with walking ability (Table 3). Walking ability was not related to dystonia, abnormal muscle tone, presence of breathing abnormalities, stereotypic hand movement, regression of hand function, or scoliosis. Multivariate analysis revealed that walking ability was only significantly correlated with meaningful words ($P = 0.0003$; odds ratio = 15.872). Even when we excluded gene effects, acquisition of meaningful words was the only robustly significant factor associated with walking ability over 10 years of age. Microcephaly, BMI, dystonia, scoliosis, and crawling were not correlated with walking ability over 10 years of age (Table 4).

4. Discussion

RTT, a neurodevelopmental disorder predominantly affecting females, has been characterized by apparently normal initial development followed by frank regression of fine motor and communication skills, typically between 6 and 18 months of age [13]. Our univariate analysis revealed that walking ability was correlated with crawling, meaningful word acquisition, microcephaly, *MECP2* mutation type, and BMI. Multivariate analysis revealed that only meaningful word acquisition was robustly related to walking ability when patients were over 10 years old. A previous study reported that walking was delayed in 30/38 RTT patients (79%); of these, 18 patients could not crawl until 4 years of age [14]. Furthermore, 55% of patients began to walk without having acquired the ability to crawl [14]. Another study reported that patients that had obtained meaningful words began crawling and walking significantly earlier than those without meaningful word acquisition, using univariate analysis [15]. Never acquiring the ability to walk is reported to be related to the early loss of language [11]. The abnormal acquisition of early skills is reported to be in accord with a marked decrease in head size beginning in early postnatal life [16], and the current data support these previous findings. In the current study, acquisition of meaningful words was a robust factor related to various symptoms or functions,

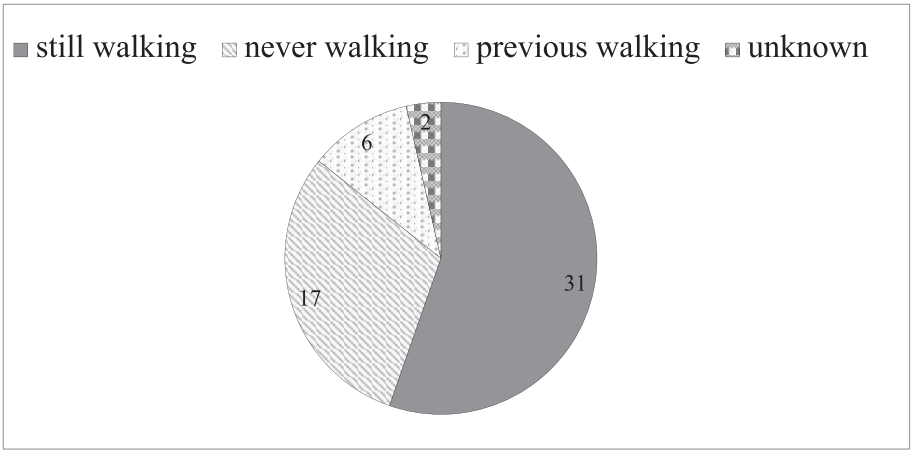

Fig. 3. Division of patients into four groups based on acquired and sustained walking abilities over 10 years of age We divided patients into four groups based on acquired and sustained walking abilities over 10 years of age: currently able to walk, previously able to walk but cannot walk at present, never able to walk, and unknown. Of 56 patients over 10 years old, 31 were currently able to walk, 17 patients never learned to walk, six patients had lost the ability to walk, and two patients had unknown walking status.

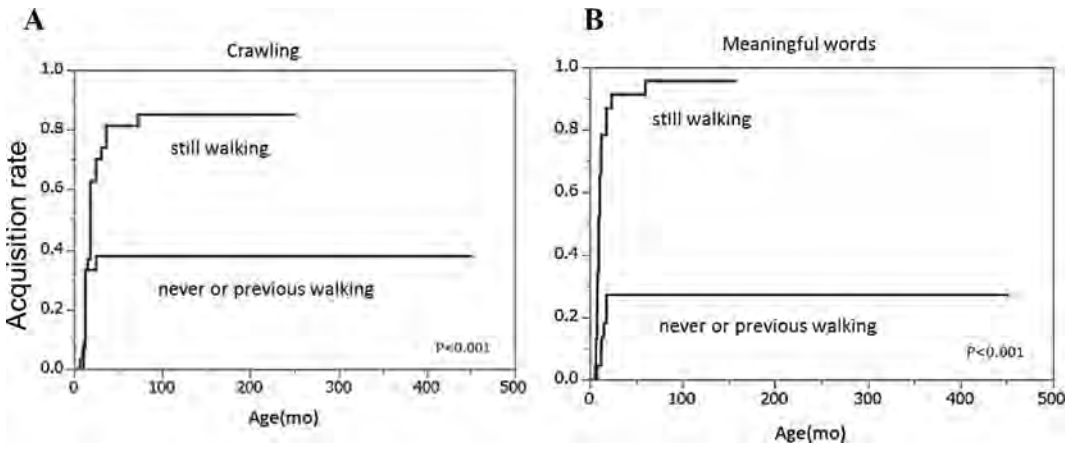

Fig. 4. Relationships between walking ability, crawling ability, and acquisition of meaningful words over 10 years of age For patients over 10 years old, walking ability was significantly different between those who acquired crawling and those who did not acquire crawling ($P < 0.001$) (A). Walking ability was significantly different between patients who acquired meaningful words and those who did not acquire meaningful words ($P < 0.001$) (B), Kaplan–Meier method. We divided participants into two groups depending on walking prognosis, as follows: participants who were still walking after 10 years of age, and participants who were either able to walk then lost walking ability or who were never able to walk (Fig. 3).

Table 3
Univariate analysis of walking ability and other factors over 10 years of age.

Factors	All patients with RTT		Excluding the patients with R306C, R133C, R294X variants	
	Odds ratio	P-value	Odds ratio	P-value
Meaningful words	7.125	<0.0001	7.959	0.0001
Microcephaly	0.265	0.005	0.261	0.0071
Dystonia	0.833	0.7357	0.831	0.7617
Scoliosis	0.778	0.6572	0.786	0.6905
BMI	1.279	0.0018	1.372	0.0019

Walking ability was significantly correlated with the acquisition of meaningful words, microcephaly and BMI; $P < 0.0001$, $P = 0005$, $P = 0.0018$, respectively. Lower BMI was negatively correlated with walking ability. BMI: Body Mass Index

Table 4
Multivariate analysis of walking ability and other factors over 10 years of age.

Factor	All patients with RTT		Excluding the patients with R306C, R133C, R294X variants	
	Odds ratio	P-value	Odds ratio	P-value
Meaningful words	15.872	0.0003	16.506	0.0015
Microcephaly	1.366	0.7132	1.706	0.5877
Dystonia	0.526	0.4024	0.685	0.6623
Scoliosis	0.758	0.7357	0.918	0.921
BMI	1.192	0.1186	1.257	0.1452

Walking ability was significantly correlated with the acquisition of meaningful words ($P = 0.0003$; odds ratio = 15.872). Although we excluded the effects of genes, meaningful word acquisition was significantly correlated with walking ability.

including cortical ability (microcephaly), growth/nutrition (BMI), motor ability (crawling), and genotype.

Witt-Engerström et al. reported that, on the basis of acquired and sustained walking ability, patients with RTT aged 22–44 years could be divided into three groups: currently walking, previously walking, and never able to walk [12]. Dystonic signs were most common among patients who were previously able to walk then became unable to walk. Early progression of scoliosis and weakness were most prevalent among patients who were never able to walk [12]. Extrapyramidal signs, including stereotypic hand movements, gait disturbance, bruxism, bradykinesia, hypomimia, scoliosis, rigidity and dystonia, have been observed in almost all patients with RTT, affecting the daily lives and walking ability of patients [17,18]. We divided patients over 10 years of age into four groups (Fig. 3). Walking ability was related to meaningful words, but dystonic signs and scoliosis were not related to walking ability. The discrepancy between the current findings and the previous report may have arisen because the diagnosis of dystonia was not reported in our study [12]. Data regarding dystonia were

collected in 67 of 100 cases (67%). Among these 67 cases, the presence of dystonia in patients with RTT was reported in 20 cases (29.9%). Over 10 years, data regarding dystonia were collected in 42 of 56 cases (75%). Among these 42 cases, dystonia was reported in 14 cases (33.3%). It is possible that the diagnosis of dystonia is difficult in some cases, and our database did not include the detail of the neurological examination of extrapyramidal signs. Unfortunately, we also did not evaluate the severity of scoliosis in the current study.

Our study confirmed the importance of genotypes associated with severe and mild phenotypes [3–5]. Tarquine et al. reported that typical RTT was associated with more severely affected growth (height, weight, head circumference, and BMI) than atypical RTT. Decreased growth, including body weight, height and microcephaly, was associated with more impaired development, higher disease severity, and specific MECP2 mutations (pre-C-terminal truncation, large deletion, T158M, R168X, R255X, and R270X) [16]. In previous reports, the mutations T158M, R255X and R168X, and R270X have generally been associated with more severe phenotypes, while R306C, R133C, R294X and 3′ truncations have been associated with less severe disease [3–5]. Patients with R306C, R133C, R294X and 3′ truncations are reported to acquire more gross motor skills and lose fewer skills, particularly in fine motor and expressive language abilities [6,7]. However specific mutations may not be the only determinant of severity within specific individuals due to the existence of other factors, such as X-chromosome inactivation, genetic background (the interplay of other genetic variations), and distribution of abnormal genes in specific brain regions [3,5,19.20]. Mutation type has some effect on the phenotypic manifestation of RTT, and the pattern of X inactivation is thought to determine phenotypic severity [21]. MECP2 interacts with a wide variety of cofactors. The intrinsically disordered nature of MECP2 permits a high degree of structural flexibility, allowing MECP2 to interact with many diverse protein partners. MECP2 utilizes a variety of mechanisms to regulate gene expression, which is dependent on the proteins with which it interacts at any given time. The clinical variability of these mutation suggests that it plays a major role in the function of MECP2 protein [22].

The database used in this study included information provided by parents and caregivers of RTT patients, with confirmation by a pediatric neurologist. Furthermore, 92 of 100 (92%) RTT participants underwent gene testing. Using a Japanese database, our results revealed that acquisition of meaningful words was the only factor that was robustly and significantly correlated with walking ability over 10 years. However, microcephaly, dystonia, scoliosis and BMI were not correlated with walking abilities in the multivariate analysis.

5. Limitations of this study

Several limitations of this study may should be considered in interpreting the results. The principal limitation was the relatively small sample size of the study, limiting the generalizability of the results. Our study also used a cross-sectional design. However, our study also had several unique features, including collaborative study of parents or caregivers, and direct examination by a pediatric neurologist. In most previous studies, data were derived from questionnaires without direct assessment of participants by clinicians experienced in the diagnosis of RTT. In addition, we performed multivariate analysis, because many factors may be tightly linked with other factors.

In conclusion, our findings may be useful for informing the development of early intervention methods, and the planning of comprehensive treatment for young infants with RTT. The acquisition of meaningful words was only significantly correlated with walking ability over 10 years of age among patients with RTT.

Author contributions

All authors have been involved in drafting or revising the manuscript, have given final approval, and agree to be accountable for all aspects of the work involved. Each author's individual participation is outlined below. TS, Shin N, ST, TM, and MI did the conceptualization and design of the study and acquisition, analysis, and interpretation of the data. MK and TK did the statistical analysis of the data. Tetsu T, KY, SN, TT, YY, YK, and CH performed the follow-up examinations and interpretation of the data.

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Conflict of interest

None of the authors have conflicts of interest to declare.

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CLINICAL REPORT

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MeCP2_e2 partially compensates for lack of MeCP2_e1: A male case of Rett syndrome

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Abstract

Background: Rett syndrome (RTT) is a neurodevelopmental disorder that predominantly affects girls. Its causative gene is the X-linked *MECP2* encoding the methyl-CpG-binding protein 2 (MeCP2). The gene comprises four exons and generates two isoforms, namely *MECP2_e1* and *MECP2_e2*. However, it remains unclear whether both MeCP2 isoforms have similar function in the brain.

Methods: We report a case of a boy with typical RTT. Male cases with *MECP2* variants have been considered inviable, but somatic mosaicism of the variants can cause RTT in males. Whole-exome sequencing was performed to search for the genetic background.

Results: A novel nonsense and mosaic variant was identified in exon 1 of *MECP2*, and the variant allele fraction (VAF) was 28%. Our patient had the same level of VAF as that in reported male cases with mosaic variants in *MECP2* exon 3 or 4, but manifested RTT symptoms that were milder in severity compared to those in these patients.

Conclusion: This is probably because the variants in *MECP2* exon 3 or 4 disrupt both isoforms of MeCP2, whereas the variant in exon 1, as presented in this study, disrupts only MeCP2_e1 but not MeCP2_e2. Therefore, our findings indicate that MeCP2_e2 may partially compensate for a deficiency in MeCP2_e1.

KEYWORDS

male with Rett syndrome, MeCP2 isoform, MeCP2_e1, MeCP2_e2, somatic mosaicism

1 | INTRODUCTION

Rett syndrome (RTT) is a neurodevelopmental disorder primarily occurring in girls. It is caused by a loss-of-function variant in one copy of the X-linked gene *MECP2* (OMIM #300005) that encodes methyl-CpG-binding protein 2 (MeCP2). In females with typical RTT due to random X-chromosome inactivation (XCI), approximately 50% of the cells express the variant *MECP2* and the other half express the wild-type *MECP2*. Males manifesting the symptoms of typical RTT also have an *MECP2* variant that is found in females with typical RTT. These males have either an extra X-chromosome (Klinefelter syndrome) or somatic mosaicism of the variant (Kleefstra et al., 2004; Schonewolf-Greulich

et al., 2019; Schwartzman, Bernardino, Nishimura, Gomes, & Zatz, 2001; Topcu et al., 2002; Villard, 2007; Zhang et al., 2019). Evidence can be obtained from male RTT patients with somatic mosaicism of the *MECP2* variant to better understand the relationship between variant allele fractions (VAFs) and the clinical severity of RTT, because the effect of XCI on the phenotype does not need to be considered in male cases.

MECP2 comprises four exons and generates two isoforms: *MECP2_e1* and *MECP2_e2* as a result of the alternative splicing of exon 2. MeCP2_e1 is translated from a start site in exon 1, and exon 2 is skipped through alternative splicing, whereas MeCP2_e2 is translated from a start site in exon 2 (Mnatzakanian et al., 2004). In this study, we sought to investigate the case of a male RTT patient mosaic for nonsense variant in exon 1 of *MECP2* that disrupts only MeCP2_e1 but not MeCP2_e2. To examine whether MeCP2_e2 can ameliorate some neurological symptoms due to the affected MeCP2_e1 functions, we compared the clinical severity of the present case with those of other reported male cases carrying the mosaic variants that affect both MeCP2_e1 and MeCP2_e2.

2 | MATERIALS AND METHODS

2.1 | Patient background and informed consent

The patient was a young boy with typical RTT phenotype who fulfilled the diagnostic criteria for the disorder (Neul et al., 2010). He and his parents gave informed consent to participate in this study. The experimental protocols were approved by the Committee for Ethical issues at Asahikawa Medical University.

2.2 | Mutation analysis of the *MECP2*

For Sanger sequencing, their DNA was used as the template for polymerase chain reaction (PCR). Appropriate primers were used to yield DNA fragments spanning the entire *MECP2* coding region and the intron–exon boundaries (Takahashi et al., 2008). The PCR fragments were analyzed using automated sequencing. Whole-exome sequencing of the DNA was performed on a HiSeq2000 sequencer (Illumina) with 101 bp paired end reads and 6 bp index reads. Exome data processing, variant calling, and variant annotation were performed as previously described (Itoh et al., 2018). To confirm the variant identified in the Sanger and whole-exome sequencing, the DNA fragment encompassing the variation site was amplified by PCR using the primers 5′-CATCACAGCCAATGACGGGC-3′ (forward) and 5′-CATCCGCCAGCCGTGTCGTC-3′ (reverse), and it was subsequently digested with restriction endonuclease Dde I. The reaction products were then visualized through ethidium bromide staining after electrophoresis on a 2% agarose gel.

2.3 | RNA isolation and RT-PCR

To examine the expression levels of *MECP2_e1* and *MECP2_e2* isoforms, total RNA was extracted from the peripheral blood cells using the PAXgene Blood RNA Kit (QIAGEN GmbH). Reverse transcription (RT) was performed using the SuperScript First-Strand Synthesis System (Invitrogen Corporation) for generation of cDNA using 1 μg of total RNA in a 20 μl reaction. Primers were designed for simultaneous amplification of both isoforms: a forward primer in exon 1 (exon1F, 5′-GAGAGGGCTGTGGTAAAAGC-3′) and a reverse primer in exon 3 (exon3R, 5′-GATGGAGCGCCGCTGTTTGG-3′), which generated a 328-bp product for *MECP2_e1* and a 452-bp product for *MECP2_e2*. As an internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as described (Itoh et al., 2012). The PCR products were visualized by ethidium bromide staining, following electrophoresis on 2% agarose gels. The optical densities of the bands were quantified using an image analysis system and ImageJ software (National Institutes of Health; Bethesda, MD). The obtained PCR products were purified from an agarose gel and directly sequenced on an ABI 3130 Genetic Analyzer (Applied Biosystems).

3 | RESULTS

3.1 | Case report

The 9-year-old male patient was born after 39 weeks of uneventful pregnancy without asphyxia. His birth weight and head circumference were 2,865 g (−0.7 *SD*) and 33.0 cm (−0.4 *SD*), respectively. He acquired head control at 3 months of age, walked alone and spoke meaningful words at 9 months, and had no apparent developmental delay until he was about 2 years old. Thereafter, his development stagnated, and it was followed by a period of regression. He became less interested in his toys and hardly had any speech. At 3 years and 6 months, he was diagnosed with autism spectrum disorder and intellectual disability. At 4 years, his purposeful hand skills began to regress, and stereotypic movements such as hand wringing appeared. At 6 years, he lost his minimal spoken language, his gait became unsteady and wide based, and his head circumference was only 47.8 cm (−2.2 *SD*), making his postnatal microcephaly more evident. He developed epilepsy at 7 years, but his seizures were eventually controlled following treatment with carbamazepine and topiramate. The chromosomal

analysis revealed normal 46,XY karyotype. No abnormal findings were observed in brain MRI and various tests related to congenital metabolic disorders.

3.2 | Molecular studies

We performed the Sanger sequencing of *MECP2* for genetic diagnosis, but the initial analysis failed to identify the pathogenic variant of this gene. Subsequently, we performed whole-exome sequencing and identified a novel nonsense and mosaic variant in exon 1 of *MECP2*, NM_001110792.1: c.31G>T; p.(Gly11*). Deep sequencing confirmed the presence of the T allele uniquely in the patient sample at a ratio of 25 mutant T allele reads to 63 G allele reads, a VAF of 28%. The reexamination of the *MECP2* variant using Sanger sequencing confirmed the wild-type sequence, with only a small amount of the variant allele, suggesting somatic mosaicism (Figure 1a). This nonsense variant created a new Dde I restriction site. Consequently, PCR-restriction digestion analysis of the DNA obtained from the patient and his parents revealed novel fragments in addition to the estimated wild-type fragment only in the patient sample, further confirming that the mosaic variant occurred de novo (Figure 1b). RT-PCR results revealed that the variant did not affect the expression levels of both *MECP2_e1* and *MECP2_e2* (Figure 2a). Analysis of cDNA showed the presence of an abnormal transcript with the nonsense variant of exon 1 in a mosaic state (Figure 2b).

3.3 | Compensatory role of MeCP2_e2 for lack of MeCP2_e1

The nonsense variant identified in the present case affected the coding sequence of *MECP2_e1* but not of *MECP2_e2*. Based on this finding, we further examined whether MeCP2_e2 is able to ameliorate the affected MeCP2_e1 function by comparing the clinical severity of the present case with those of six previously reported RTT males carrying *MECP2* mosaic variants that affect both MeCP2_e1 and MeCP2_e2 (Table 1). This comparison revealed that the median VAFs and the age at onset of regression of the reported male patients were 25% (range 9–36) and 13 months (range 8–18), respectively, whereas in the present case with 28% VAF, the developmental problems were not noticed until the patient was 2 years old. At 9 years, he was already able to walk independently, although his balance was poor. Among the six previously reported cases, only three were able to walk with or without support.

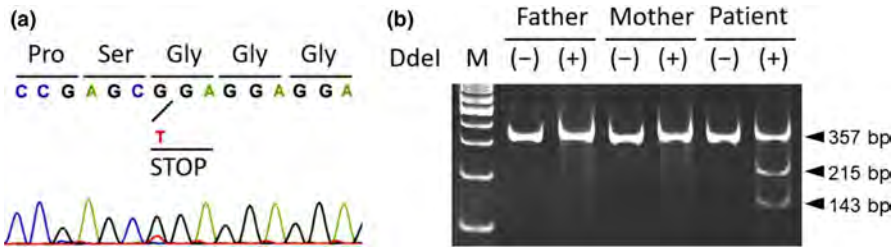

FIGURE 1 A novel mosaic variant of *MECP2* in a male patient with typical Rett syndrome. Electropherogram shows the nonsense variant in exon 1 of *MECP2* [NM_001110792.1: c.31G>T: p.(Gly11*)] in mosaicism (a). Dde I digestion of the PCR product encompassing the variation site shows additional fragments (143 and 215 bp), which resulted from the G-to-T transition creating a new Dde I restriction site in the patient but not in his parents (b). These additional fragments are observed together with the 358 bp wild-type fragment, confirming the mosaic variant in the patient

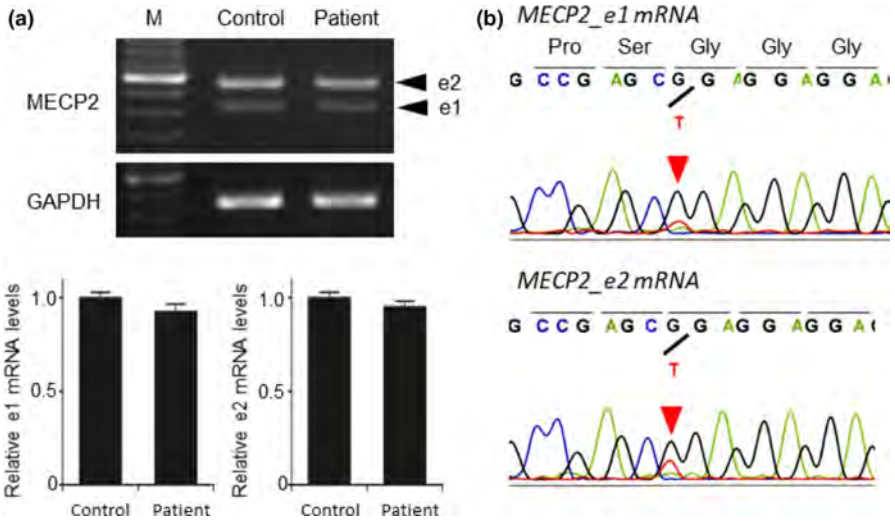

FIGURE 2 Equal amounts of *MECP2_e1* and *MECP2_e2* mRNA and abnormal transcript with the nonsense variant in a mosaic state. RT-PCR results reveal that both *MECP2_e1* and *MECP2_e2* mRNA amounts are unaffected in the patient. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control (a). Sequencing analysis of the obtained PCR products shows the presence of abnormal transcript with the nonsense variant in a mosaic state (b)

TABLE 1 Relationship between variant allele fractions and the clinical severity in males with typical Rett syndrome associated with mosaic *MECP2* variants

	Variants							
Age at evaluation (Authors)	Nucleotide change	Predicted effect on protein sequence	VAFs[a]	Onset of regression	Hand skills	Language	Gait	Stereotypy
9 years (Present case)	c.31G>T	p.(Gly11*)	28%	24 months	Poor	Lost	Poor	Present
12 years (Topcu et al., 2002)	c.808C>T	p.(Arg270*)	36%	11 months	Lost	Never	Never	Present
11 years (Kleefstra et al., 2004)	c.473G>T	p.(Thr158Met)	25%	13 months	Lost	Lost	Lost	Present
2 years (Zhang et al., 2019)	c.316C>T	p.(Arg106Trp)	26%	18 months	Never	Never	Never	Present
2 years (Zhang et al., 2019)	c.353G>A	p.(Gly118Val)	20%	13 months	Poor	Lost	Poor	Present
8 years (Schonewolf-Greulich et al., 2019)	c.1308dupT	p.(Gln437Serfs*50)	9% (15%)	18 months	Poor	Lost	Poor	Present
9 years (Schonewolf-Greulich et al., 2019)	c.808C>T	p.(Arg270*)	36% (45%)	8 months	Lost	Never	Poor	Present

[a]VAFs, variant allele fractions in blood lymphocytes (in fibroblasts).

4 | DISCUSSION

We present clinical and molecular findings in a male RTT mosaic for a novel nonsense variant of *MECP2*. Deep sequencing with a next-generation sequencing method revealed the small amount of the variant allele c.31G>T; p.(Gly11*) with 28% VAF, which the initial Sanger sequencing failed to identify. The PCR-restriction digestion analysis further confirmed the somatic mosaicism of the variant. RT-PCR results revealed that both *MECP2_e1* and *MECP2_e2* mRNA amounts were unaffected in the patient, suggesting that the nonsense variant in exon 1 of *MECP2* likely escapes nonsense-mediated mRNA decay (NMD) and does not affect transcription of *MECP2_e2*. The level of sensitivity of a premature-termination codon (PTC) - containing mRNA to NMD is multifactorial. It has been shown that mRNAs carrying PTCs in close proximity to the translation initiation AUG codon escape NMD, called the "AUG-proximity effect" (Silva, Ribeiro, Inacio, Liebhaber, & Romao, 2008). Consequently, this variant may lead to translational reinitiation at a downstream AUG codon producing an N-terminally truncated protein functionally distinct from wild-type MeCP2_e1, but does not affect the translation of *MECP2_e2*.

The majority of the RTT-associated *MECP2* variants are located in exons 3 and 4, which simultaneously disrupt both the MeCP2_e1 and MeCP2_e2 isoforms. RTT associated with exon 1 variants of *MECP2* is rare, and its detection rate is 8.1% in patients with typical or atypical RTT (Saunders, Minassian, Chow, Zhao, & Vincent, 2009). However, exon 2 variants that exclusively affect MeCP2_e2 have never been identified in RTT, suggesting that MeCP2_e2 does not have an essential function in the brain. Supporting this notion, a mouse model with a deletion in *MECP2* exon 2 failed to recapitulate the neurologic symptoms characteristic of RTT (Itoh et al., 2012). In this study, we presented the nonsense variant in exon 1 of *MECP2* that disrupted MeCP2_e1 but not MeCP2_e2. Notably, however, we examined the variant using DNA extracted from peripheral blood leukocytes, so that it is uncertain whether the VAF in brain will be equivalent to that observed in the present study. Nonetheless, this study demonstrated that a male carrying an *MECP2* exon 1 mosaic variant and expressing 28% less MeCP2_e1 in blood than normal individuals exhibited the typical RTT phenotype, indicating that even a mild reduction of MeCP2_e1 is sufficient to cause RTT.

In a previous study using the isoform-specific knockout mice in which Mecp2_e1 was lacking while the expression of Mecp2_e2 was preserved, the neurologic deficits of RTT were recapitulated (Yasui et al., 2014). The study implied that an RTT phenotype may occur even in the presence of MeCP2_e2, which is unable to compensate for the lack of MeCP2_e1. Nevertheless, recent animal studies have revealed the partial rescue of Rett-like symptoms in Mecp2-null mice through the reexpression of Mecp2_e2 (Jugloff et al., 2008; Kerr et al., 2012). However, there had been no clinical evidence whether MeCP2_e2 is able to ameliorate some neurological symptoms due to the affected MeCP2_e1 functions. Genotype–phenotype correlations are difficult to make in female RTT patients because of the differences in XCI. Examination of male RTT patients with somatic mosaicism of the *MECP2* variant allowed us to assess the relationship between VAFs and clinical severity. Comparison of the clinical severity of the present case involving the *MECP2_e1*-specific variant

with those of other reported male cases with mosaic variants that affect both MeCP2_e1 and MeCP2_e2 revealed that the present case had a milder phenotype even though the VAFs were almost the same (Table 1). In conclusion, this study is the first to present clinical and molecular evidence that MeCP2_e2 may partially compensate for the deficiency of MeCP2_e1, although further functional studies are needed.

ACKNOWLEDGMENTS

We sincerely thank the patient and his parents, whose help and participation made this work possible.

CONFLICT OF INTEREST

None of the authors declare any conflict of interest related to this study.

AUTHOR CONTRIBUTIONS

RT and ST conceived and planned the study, and drafted the manuscript. MI performed whole-exome sequencing. RT, ST, MK, RT, and NS performed genetic analysis. YT, YI, and NS acquired clinical phenotype data. All authors provided important feedback on the analysis and manuscript, and approved the final version.

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シンポジウム 7：患者会と進める日本レット症候群研究・治療の現状

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企画・趣旨のねらい

レット症候群（RTT）は，主に女児に発症し，乳児期早期から筋緊張低下，自閉傾向，四つ這い，歩行などのロコモーション障害，重度の知的障害などが出現する．その後，目的をもった手の運動機能の消失，手の常同運動が出現するユニークな病態を示す．遺伝子のメチル化，および転写抑制に関わる MECP2 遺伝子の異常が発見され注目を集めており，米国，ヨーロッパ，オーストラリアでは大規模なデータベースが作成され研究が進んできた．2017 年の Nature Reviews によると，それまでに 4,000 以上の医学論文，25 以上の新規治療法開発の臨床治験が進行中または一部で治験が終了したことが報告され，発達障害解明の鍵となる症候群である．その後，MECP2 遺伝子の重複症候群も報告され新しい展開が期待されている．日本では医学関係者が，厚生労働省，AMED などの公的資金を獲得し，3 つの患者家族会と協働で RTT のデータベースを作成し，約 150 例が登録された．RTT の自然歴を知る事は重要であるが，本取り組みの認知度が未だ低く，登録状況が進んでいないのが現状である．病態解明・今後の新規治療法開発を視野に入れて，6 人の演者から本邦での取り組みの現状，世界の動向解説，将来へ向けての展望について発表頂く．

S7-1
Rett syndrome, Overview —past to future—
（レット症候群 overview —これまでとこれから—）

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レット症候群は主に女児にみられる，乳児期から始まる姿勢や協調運動の障害，対人関係の障害，知的発達障害，特徴的な常同運動，退行を特徴とし，年齢依存性にてんかんなどの症状を呈する疾患である．1966 年に A. Rett が初めて報告し，1983 年に B. Hagberg によって 35 例の詳細な報告により疾患単位として確立され，臨床研究が進められた．2000 年頃までに病期分類や重症度分類などが作られ，臨床評価に使われている．本邦の全国調査では，国内の有病率は約 0.008％で諸外国と差はなく，20 歳以下女性の患者数は約 1,000 人程度と推定されている．有効な治療法はいまだなく，多科にわたる診療と多方面の取組みが必要であり，早期の医療・療育介入が求められる．

1999 年に MECP2 遺伝子が原因遺伝子として報告され，その後非典型例で CDKL5 遺伝子と FOXG1 遺伝子の原因遺伝子が報告された．2010 年の改正診断基準によると，典型的レット症候群の約 95％に MECP2 遺伝子変異があることが分かってきた．早期診断には，臨床診断に基づいた遺伝子検査が有効であると考えられている．2011 年より患者データベースの運用が行われている．自然歴研究や治験を推進することを目的とし，患者団体とともに進めているプロジェクトである．現在，約 160 例の登録があり，欧米豪を中心とした世界的なネットワークを出来つつある．

一方，2001 年に疾患モデルマウスが開発され，基礎研究が進んだ．現在では，多種の遺伝子改変マウスによる病態研究や分子生物学的研究が進められ，最も基礎研究が進んでいる発達障害の一つである．近年の再生医療や遺伝子編集の技術的進展は，レット症候群を含めた遺伝性発達障害の展望を期待させる成果を上げている．本項では，レット症候群の歴史を振り返り，基礎研究を背景とした今後の展望について議論したい．

シンポジウム 7：患者会と進める日本レット症候群研究・治療の現状

S7-2
The impact of MECP2 loss- or gain-of-function on clinical presentations —Rett syndrome and MECP2 duplication syndrome— （MECP2 遺伝子変異と臨床像との関連 —機能喪失型（レット症候群）と 機能獲得型（MECP2 重複症候群）—）

高橋　悟（Satoru Takahashi）
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MECP2 遺伝子は X 染色体（Xq28）上に位置し，“エピジェネティクス”と呼ばれる遺伝子発現制御における重要な分子をコードしている．その発現量は，胎生期には低く，生後に神経細胞の成熟に伴って高くなる．MECP2 の機能喪失によりレット症候群が，また機能亢進により MECP2 重複症候群が発症する．このように，神経細胞の成熟および脳機能維持のためには MECP2 発現量のコントロールが重要である．

レット症候群は，主に女児に発症し，一旦獲得した運動機能・言語能力を失うといった退行を特徴とする神経発達障害である．乳児期には異常に気づかれず，退行は 1 歳以降に急速に進行することが多い．典型例の 90–95％で，MECP2 に機能喪失性変異が同定される．同じ遺伝子変異を有する患者間でも，臨床的重症度は一致しないことがあり，中枢神経系内での X 染色体の不活化パターンの多様性が臨床像に影響を与えている．一方，MECP2 重複症候群は，主に男児に発症し，種々の神経症状が年齢依存性に出現する．2017 年に行われた本邦初の全国調査から 24 例の臨床的特徴がまとめられ，早期診断に役立つ症状として，乳児期から明らかとなる筋緊張低下，運動発達遅滞，特徴ある顔貌，繰り返す感染症が抽出された．てんかんはおよそ 70％の患者で認められたが，その発症は比較的遅く幼児期以降であることが多かった．家族内発症する場合，同胞患者間の臨床像はほぼ同一である．一方，保因者である母親は，重複を有する X 染色体が選択的に不活化されており，無症状であることが多い．

両疾患のモデル動物を用いた研究では，MECP2 発現量を正常化すると神経症状は可逆的に改善することが示されている．対症療法から治癒を目指した薬物治療・遺伝子治療へ向けて，MECP2 発現量のコントロールが重要な課題である．

S7-3
Role of Mecp2-null mouse model of Rett syndrome in understanding the pathophysiology and in identifying potential therapies （レット症候群モデルマウスを用いた病態解明と 治療応用への展望）

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レット症候群（RTT）は，主に女児で発症し，Methyl-CpG-binding protein 2（MeCP2）遺伝子の変異によって引き起こされる重度の神経発達障害である．RTT は，重度の知的障害，自閉性行動に加えて，てんかん，不随意運動，自律神経障害，心電図異常，睡眠障害等の多様な神経症状を特徴とする．久留米大学小児科では，長年，RTT の診断・治療に携わると同時に，病態モデルによる基礎研究を行ってきた．特に近年，特定の遺伝子異常によって自閉傾向，重度の精神遅滞を呈する RTT の病態解析は，発達障害をはじめとする精神神経疾患の発症に共通するメカニズムの解明につながると考えられ，大きな関心を集めている．実際，様々な MeCP2 変異マウスは，疾患モデル動物として様々な症状を模倣し，様々な病態を理解する良いモデルとなると考えられている．RTT モデル（MeCP2 欠損）マウスは，生後 4 週目頃までは正常に発育する一方で，6 週目以降，体重増加が遅れ，呼吸異常や不整脈などの症状をともない，10 週目までにはそのほとんどが死亡する．臨床上，RTT では，自律神経の異常による不整脈や呼吸異常によるとされる突然死が問題になっており，我々は，これまで，MeCP2 欠損マウスの早期死亡に関連するとされる心臓機能や呼吸の異常に着目して研究を行ってきた．また，80％以上の RTT 患者で合併する睡眠障害は，本人の QOL 低下に加え，介護者の負担を増加させ，臨床における問題の一つである．そこで，我々は，MeCP2 欠損マウスにおいて，明暗期における活動量や睡眠・覚醒解析を行い，睡眠障害に関連する病態メカニズムの解析も進めている．本講演では，RTT モデルマウスにおける早期死亡や睡眠研究に関する我々の研究成果を中心に，病態メカニズムに関する最近の知見を紹介し，今後の治療研究における応用について考えてみたい．

シンポジウム 7：患者会と進める日本レット症候群研究・治療の現状

S7-4
Review of my clinical experience of Rett syndrome after the creation of a guidebook（レット症候群の臨床的研究 ガイドブック作成から見えてきたこと）

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レット症候群は，Andreas Rett により 1966 年に報告された女児のみが罹患する疾患です．当初は原因不明でしたが，手の常同運動という特徴的な症状があり，その他の様々な臨床症状を組み合わせて診断する診断基準が，何回も改訂されてきました．1999 年に原因遺伝子が MECP2 であると判明しました．最新の診断基準は 2010 年に作成されたもので，レット症候群と診断された患者 819 人のうち，MECP2 遺伝子変異陽性例に特異的に認めた症状を整理しました．これにより，本質的で重要な症状が明確になり，また診断要件を陽性とする判断基準を明確にしたため，診断しやすくなりました．私たちは 2015 年に診療ガイドブックを出版しました．このガイドブックの内容は，まず，レット症候群の歴史，診断基準，遺伝子，病態についてまとめ，主要な症状についてその概説と病態，また対処法について最新の文献にあたりながら記載しました．また，診療上重要な社会福祉資源や研究中の治療についても触れています．レット症候群では，上肢機能と言語機能の退行と歩行障害，てんかんや発達障害，嚥下障害といった神経症状以外に，側弯や便秘・呑気，歯ぎしり，不整脈など，多彩です．レット症候群の根本的な治療はなく，対症療法しかありません．とはいえ，こうした様々な症状を広く視野に入れて診療することは容易ではありません．私たちの目標は，臨床家にとって，現時点で最善の情報を集めて，診療の手助けになることでした．ガイドブックを出版して 5 年，現在も根本治療はありません．MECP2 遺伝子が過剰に発現した場合は，MECP2 重複症候群という別の重度神経障害を呈する疾患と同じ状態になります．レット症候群を治療するためには，変異 MECP2 遺伝子が存在する X 染色体が不活化されていない細胞のみに限定して，適正な量を発現するように調整した正常 MECP2 遺伝子を導入しなければならず，非常に難易度の高い治療となります．アメリカでは，“care today, cure tomorrow”と言って，今は治療法はないけれど，希望を捨てずにできることをして待つことが言われています．本講演では，ガイドブックを作成した後の臨床経験について，レビューします．

S7-5
Research and development of new treatment using Ghrelin for Rett syndrome —Including reviews of treatment development around the world—（グレリンを用いたレット症候群の新規治療法開発 —世界の治療開発のレビューを含めて—）

弓削康太郎（Kotaro Yuge）
久留米大学小児科

世界中でレット症候群に対する新規治療法の研究が行なわれているが未だ有効な治療薬はない．国内では L-ドーパ，ドパミンアゴニストなどが使用された経験はあるが，有効性を証明する事はできていない．海外では，2017 年の Review で（A）神経伝達物質の異常，（B）成長因子，成長，神経栄養因子，代謝異常，ミトコンドリア異常，（C）MECP2 遺伝子，RNA，蛋白をターゲットとする治療の可能性として 25 の治療薬候補があげられ，その一部は試験が完了している（Leonard et al. 2017）．この中では，Trofinetide（terminal tripeptide of IGF-1）の高用量投与において有効な可能性が残され，第 3 相試験が検討されている（Glaze et al. 2019）．しかし，レット症候群に対して明らかに有効性が証明されたものはなく承認された医薬品はない．今回，我々はレット症候群に対する新規治療法としてグレリンの研究開発を進めているため現状を報告する．グレリンは日本の研究者により発見された 28 残基のペプチドホルモンで，主に胃で産生され血液循環を経て全身へ，神経系へは血液脳関門を通り視床下部・脳幹へ働き摂食，消化，代謝，循環へ影響を及ぼす．本研究はグレリンの脳内での働きに注目して始まった．開発の経緯としては，基礎研究ではモデルマウスにて脳内グレリン含量・血中グレリン濃度の低下を認め，臨床研究では 10 歳以降の患者で血中濃度の低下，グレリン投与によるジストニア・振戦・自律神経症状などの改善を認めた．これらより医師主導治験を実施するために医薬品医療機器総合機構（PMDA）との対面助言を行い，プロトコル概要・非臨床安全性試験について合意を得た．現在の日本医療研究開発機構（AMED）の支援を受けた取り組みと今後の展望について報告する．

シンポジウム 7：患者会と進める日本レット症候群研究・治療の現状

S7-6
Activities of the patient association aiming at the development of treatment methods
（治療法開発を目指した患者会の活動）

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NPO 法人レット症候群支援機構

私が団体を設立した 10 年前，患者団体の大きな役割は，患者交流であった．

しかし近年，インターネットや SNS 等の普及により，患者同士の交流は日本のみならず世界中で交流する事や，情報交換をする事が可能となっている．私は 2011 年 4 月に NPO 法人レット症候群支援機構という，患者団体がレット症候群の研究を支援し治療法開発の一端を担うという目的を掲げて NPO 法人を設立した．他の団体と大きく違ったのが，患者交流を主な目的にはせずに，研究支援や患者同士交流だけではなく研究者との交流を目的に掲げたという事．NPO 法人という形態をとる事で事業として，研究支援やシンポジウムの開催，情報収集を行っている．この法人の活動を支えるのは登録をしている会員．現在会員数は 82 名，そのうち患者家族は 68 名で年会費を収めたり，情報収集，募金活動，啓発活動，様々な活動を行っている．それらの活動の結果，世間一般の方や企業からの寄付金でレット症候群の研究支援を行っている．現在で研究の支援金は累計 1,200 万円になる．研究支援を行う一方で日本全国様々な研究者とも交流を重ね，常に患者団体の新しい役割を検討してきた．その一つが研究班と共同で築き上げたレット症候群患者データベースだ．課題はまだまだあるものの，研究者と患者の間を繋ぎ双方にメリットが出る活動を今後も継続していく為に私達がしてきた事と出来る事を紹介したい．

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