Diagnosis and Genetic Counseling for Mitochondrial Disease at the Institute of Medical Genetics, Tokyo Women's Medical University

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Diagnosis and Genetic Counseling for Mitochondrial Disease at the Institute of Medical Genetics, Tokyo Women's Medical University

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We performed a retrospective review of medical records of patients seeking genetic counseling for mitochondrial disease at our clinic between 2004 and 2015. Of a total of 31 adult subjects (male to female ratio= 1 : 2.1; mean age at first visit 40 years), 27 (87.1 %) underwent genetic testing. The results of the genetic testing revealed a gene mutation causing the mitochondrial disease in 20/27 (74.1 %) subjects. The mutation detection ratio in blood samples from symptomatic subjects was 17/22 (77.3 %). The mutation detection ratio differed according to family history of mitochondrial disease. Of the 31 subjects, 13 (42.0 %) learned that they had at-risk family members based on the results of their own genetic testing. Eight female subjects who were single or without a child or pregnant underwent genetic counseling to obtain a precise diagnosis and identify the genetic cause based on a detailed family history and other medical information. The genetic cause in these cases was usually not identified. Even in the case of a precise diagnosis, there may not be sufficient natural history information, which makes presymptomatic and prenatal genetic testing difficult. For subjects with mitochondrial disease and their families, uncertainty about their future leads to great emotional stress. Our findings indicate the importance of providing clear information about the disease and its uncertainties, empathetically listening to the patients, and assisting patients with the process of adapting to their situation. Long-term follow-up is necessary to track changes in symptoms or in family situations, as well as to detect new cases.

Key Words: mitochondrial disease, maternal inheritance, genetic counseling

Introduction

Mitochondrial disease is a clinically heterogeneous group of disorders resulting from dysfunction of the mitochondrial respiratory chain[1)]. Mitochondria are present in all eukaryotic cells except red blood cells and generate most of the adenosine triphosphate in the cell. Mitochondrial dysfunction is an important cause of human disease, especially in organs and tissues with high energy requirements. Each human cell contains hundreds of mitochondria that have approximately 5 to 10 copies of circular mitochondrial DNA (mtDNA) comprising 16,568 bp. The mtDNA encodes 37 genes: 13 for subunits of respiratory complexes I, III, IV, and V; 22 for mitochondrial transfer RNA; and 2 for ribosomal RNA. When an egg cell is fertilized by sperm, the paternal mitochondria are

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marked with ubiquitin to select them for later destruction inside the embryo. Therefore, mtDNA usually come from only the egg. The presence of identical copies of mtDNA in a cell or tissue is called "homoplasmy"[2]. In contrast, the presence of more than one type of mtDNA in a cell or tissue is called "heteroplasmy". When the percentage of mutated mtDNA exceeds a critical threshold, mitochondrial disease results[3)4)]. As the percentage of mutated mtDNA varies among organs and tissues, detection of mutated mtDNA in blood specimens may be difficult, and it is thus sometimes necessary to obtain specimens of the muscle or affected tissue. The common A3243G mutation occurs in coding region of a mitochondrial transfer RNA gene. This mutation is associated with several mitochondrial diseases, including mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) and diabetes mellitus[5]. The A3243G point mutation resulted in severe respiratory chain deficiency. Another common mutation in the MTTE gene, T14709C, is associated with maternally inherited diabetes and deafness[6].

The vast majority of the 1,500 proteins composing mitochondria are synthesized from nuclear gene transcripts, but 13 essential subunits are encoded by mtDNA. Therefore, the genetic cause of mitochondrial disease may be a defect of the nuclear DNA or the mtDNA. The defect may be inherited in autosomal recessive, autosomal dominant, or X-linked manner, with maternal inheritance[7]. Mitochondrial disease may involve multiple organs, and present at any age with a varied symptomatology as well as a varied clinical course. Despite pathologic, biochemical, and genetic testing, the genetic cause is detected in only half of the patients with a suspected mitochondrial defect. If the genetic cause of the disease is not identified, genetic counseling is difficult due to genetic heterogeneity. Here we report a summary of our genetic counseling experience of patients with mitochondrial disease, and discuss critical issues.

Subjects and Methods

Subjects were 31 adults who visited the Institute of Medical Genetics, Tokyo Women's Medical University between 2004 and 2015, and underwent genetic counseling or medical examination for mitochondrial disease. Data regarding age, sex, consultation motive, discussions, symptoms, family history, and the results of genetic testing, were retrospectively collected from the medical records. All studies were conducted in accordance with the Declaration of Helsinki and approved by the ethics committees.

Results

The flow of genetic counseling related to mitochondrial disease throughout this study is shown in **Fig. 1**. There were 31 subjects (10 men, 21 women) with a mean age at first visit of 40 years (range 21-61) (**Table 1, 2**). The consultation motive was "diagnosis of symptomatic case" in 22 (71.0 %) cases, "presymptomatic genetic testing" in 5 (16.1 %), "provision of disease information" in 3 (9.7 %), and "estimation of recurrent risk in family" in 1 (3.2 %). Of the 27 subjects with symptoms or who were presymptomatic, a mutation was detected in 20 (74.1 %) subjects, including two presymptomatic cases, but no mutation was detected in 7 (25.9 %) cases, including two presymptomatic cases. Specimens collected for genetic testing were only peripheral blood in 22, peripheral blood and skeletal muscle in 2, skeletal muscle in 2, and peripheral blood and kidney in 1. The mutation detection rate of genetic testing using only peripheral blood was 19/22 (86.4 %), and the median age at genetic testing was 42 years (21-58). The mutation detection rate of genetic testing using another affected tissue was 1/5 (20.0 %). If no information of familial mutation was available, we first examined the 3243 mtDNA point mutation. If no 3243 mtDNA mutation was detected, we used other detection methods, including mtDNA full-sequencing, long polymerase chain reaction, or Southern blot analysis for mtDNA copy variants.

Gene mutations were detected in cases 18 of 23 symptomatic subjects (excluding 4 subjects examined before symptoms manifested) with a family history consistent with maternal inheritance (e.g., similar diseases in children and mother, maternal grandmother, maternal grandparents, siblings, or women), and gene mutations were not detected in sporadic cases, cases No. 17-21, indicating a family history not consistent with maternal inheritance. The final gene mutation detection ratio for each symptom was as follows: diabetes+deafness 93.3 % (14/

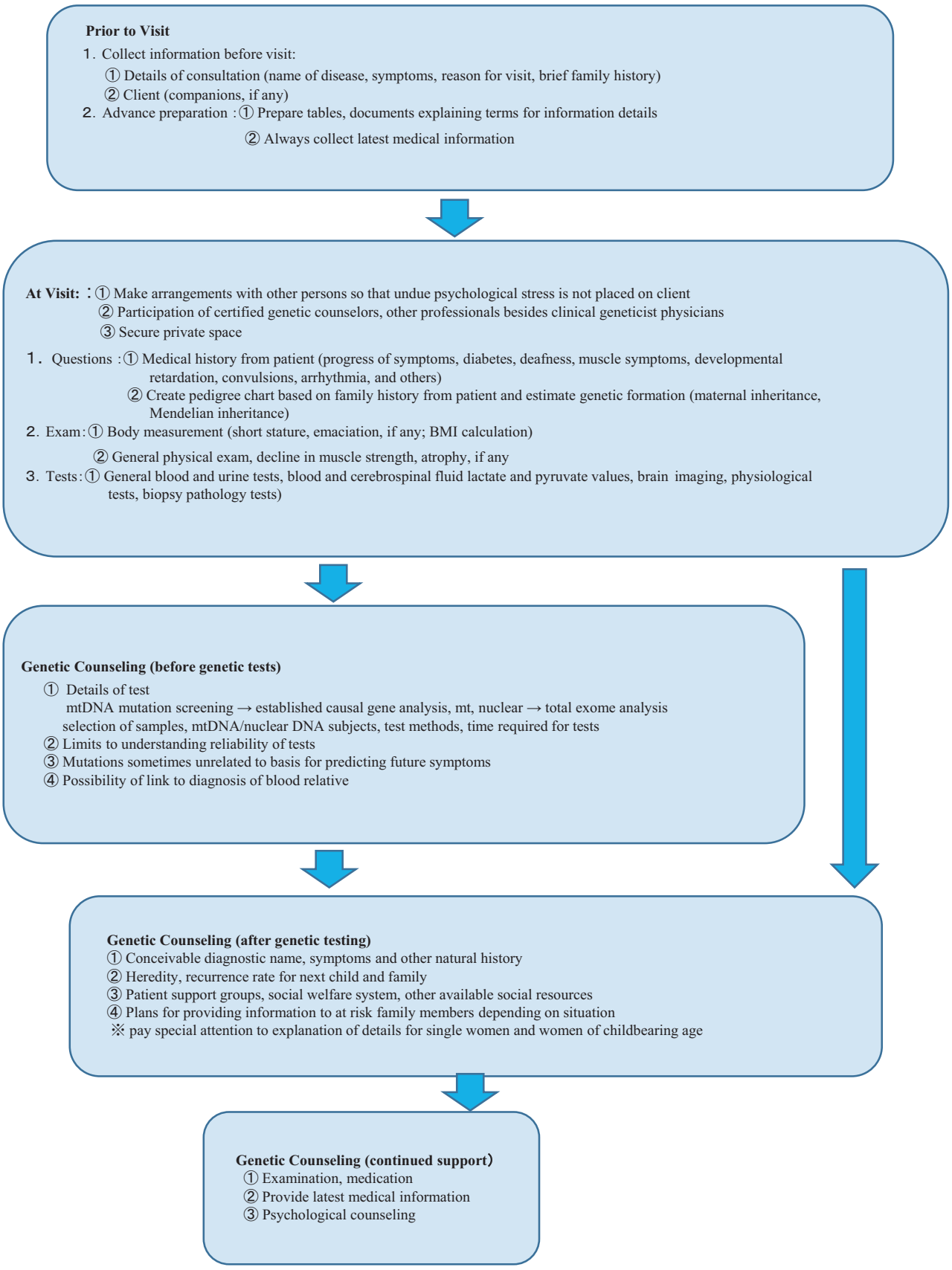

Fig. 1 Flow of treatment and genetic counseling for mitochondrial diseases

Table 1 List of Clients

No.	Age	Sex	Details of consultation	Familial estimate person at risk	Noteworthy status	Symptoms			
						Diabetes	Onset age of diabetes	Deafness	Others
1	29	M	DC	Confirmed	…	+	24	-	Tinnitus, decreased muscle force
2	36	F	DC	Confirmed	…	+	35	+	Developmental retardation, low birth weight, cerebral palsy, headache
3	47	F	DC	Confirmed	…	+	47	+	Renal symptoms
4	58	M	DC	None	…	+	31	-	Decreased muscle strength, renal symptoms, retinopathy, childhood acetonemic vomiting
5	35	F	DC	Likely	…	+	19	+	…
6	22	F	DC	None	Unmarried	+	22	+	Short stature
7	26	F	DC	None	No children	+	21	-	Tinnitus
8	47	M	DC	None	…	+	27	+	Tinnitus, autonomic disorder, codeine addiction
9	44	F	DC	None	…	+	32	+	Short stature, apoplectic seizures, headache
10	42	M	DC	None	…	+	27	+	Convulsions, stenocardia, unconsciousness, arrhythmia
11	47	M	DC	Likely	…	+	32	+	BMI 15 ～ 16
12	55	F	DC	Likely	…	+	25	+	…
13	58	F	DC	Confirmed	…	+	22	+	Myocardiopathy
14	57	F	DC	Likely	…	+	46	+	Renal symptoms
15	40	F	DC	None	…	+	25	+	Renal symptoms
16	57	F	DC	Likely	…	+	42	+	Short stature, emaciation, decline in muscle force
17	48	M	DC	None	…	+	43	-	Renal symptoms
18	27	M	DC	None	…	-	…	-	Convulsions, dizziness, ophthalmoplegia externa, arrhythmia
19	42	F	DC	Likely	…	-	…	-	Epilepsy, arrhythmia
20	45	M	DC	Likely	…	+	32	+	Decline in muscle strength, renal symptoms, ophthalmoplegia externa, decline in visual acuity
21	21	F	DC	None	Unmarried	-	…	-	Renal symptoms
22	32	F	DC	None	Pregnant	GDM	32	+	Tinnitus, renal symptoms, headache
23	33	F	DBO	None	No children → Pregnancy	- → +	…	-	…
24	26	M	DBO	None	…	-	…	-	…
25	27	M	DBO	None	…	-	…	-	…
26	53	F	DBO	None	…	-	…	-	…
27	33	F	DBO	None	No children	-	…	-	…
28	39	F	DBO	Likely	…	+	27	+	Depression
29	29	F	IP	None	Unmarried	+	18	+	…
30	25	F	IP	None	No children	-	…	-	Arrhythmia
31	61	F	RRE	Likely	…	-	…	-	…

BMI, body mass index; DBO, diagnosis before onset; DC, diagnosis confirmed; F, female; GDM, gestational diabetes mellitus; IP, information provided; M, male; RRE, recurrence rate estimate.

15), diabetes 89.5 % (17/19), muscle symptoms 75.0 % (3/4), nephropathy 57.1 % (4/7), short stature 100 % (3/3), tinnitus 100 % (3/3), convulsions 33.3 % (1/3), arrhythmia 33.3 % (1/3), headache 100 % (2/2), and ophthalmoplegia externa 0 % (0/2). Median age at onset when mutations were detected and time of onset of diabetes was 27. Diabetic symptoms manifested after testing and pregnancy were confirmed in case No. 19 who was examined before symptoms of mitochondrial disease appeared and in whom the 3243 mutation was detected. When diagnosis of the subject or the proband was confirmed, there were 13 subjects (42.0 %) who had family members at risk. Of these, 4 subjects (30.8 %) in particular had family members in whom mutations were theoretically "confirmed" when the subject or their proband was diagnosed. The subjects included 8 women (25.8 %) who were single, without children or pregnant.

During genetic counseling, the clients made remarks, such as "What will happen when I get older?", "I am worried about what will happen in the future", "If I have mitochondrial disease, most likely this will be passed on to my children and I don't know what symptoms will appear". During the course of the visit, comments such as "I can handle the disease, but I don't want to pass this

Table 2 Client List (continued)

No.	Test findings			Family history	Genetic test	
	Lactate value (mg/dl) (source)	Biopsy	Other		Results	Specimen
1	16.4 (cerebrospinal fluid)	Muscle		T14709C	T14709C	Blood
2	43.8 (cerebrospinal fluid)	Muscle	…	+	A3243G	Blood
3	19.3 (blood)	Liver	…	+	A3243G	Blood
4	…	Muscle	…	+	A3243G	Muscle
5	…	…	…	+	A3243G	Blood
6	…	…	Anti-GAD antibodies <0.3	+	A3243G	Blood
7	Normal	…	Cerebellar atrophy Anti-GAD antibodies <0.3	+	A3243G	Blood
8	…	…	…	+	A3243G	Blood
9	…	…	…	+	A3243G	Blood
10	…	…	…	+	A3243G	Blood
11	…	…	…	+	A3243G	Blood
12	…	…	…	+	A3243G	Blood
13	…	…	…	+	A3243G	Blood
14	…	…	…	+	A3243G	Blood
15	…	…	…	+	A3243G	Blood
16	Normal	…	…	+	A3243G	Blood
17	…	…	…	* +/-	Unclear	Blood
18	…	RRF in muscle, various size type 1 fibers in biopsy	…	…	Unclear	Fluid, muscle
19	17.8 (cerebrospinal fluid)	Myogenic changes in muscle	Old infraction in cerebellar hemisphere	…	Unclear	Fluid, muscle
20	31 (blood)	RRF in muscle	…	…	Unclear	Muscle
21	25.7 (blood)	Mitochondrial accumulation in liver	…	…	Unclear	Blood, kidney
22	29.8 (cerebrospinal fluid)	Liver	Anti-GAD antibodies <=1.3	…	Not carried out	…
23	…	…	…	+	A3243G	Blood
24	…	…	…	+	A3243G	Blood
25	…	…	…	A3243G	Negative	Blood
26	…	…	…	A3243G	Negative	Blood
27	…	…	…	A3243G	Not carried out	…
28	…	…	…	+	Not carried out	…
29	…	…	…	+	A3243G	Blood
30	…	…	…	+	A3243G	Blood
31	…	…	…	+	Not carried out	…

+/-, maternal diabetes and paternal nephropathy; RRF, ragged red fibers.

disease to my child. I want this to stop with me", "I can accept this as long as the diagnosis for myself, but I am worried about passing this on to my child", "It is difficult for me to think that there is a 100 % chance that the child in my womb will have a mitochondrial disease. I can give birth to a healthy child, can't I? Things will be all right, won't they?", and other emotional concerns were expressed by female clients.

Discussion

In this study, gene mutations were detected in 18 (78.3 %) of 23 symptomatic subjects. In general, the mtDNA mutation ratio for each tissue differs[8] and it is well-known that the mutation rate in the blood is low. Although the affected organs and the skeletal muscle should be used as samples in genetic testing, the mutation detection ratio in this study based on analysis of symptomatic subjects using blood samples was 77.3 %. In addition, in mutation-positive symptomatic subjects, 17 peripheral blood samples (94.4 %) and 1 muscle sample (5.6 %) were obtained. In addition, the overall rate of complications of diabetes in this study was high at 71.0 % (22/31), and many subjects had histories consistent with maternally inherited diabetes and deafness (MIDD; #520000). Nonetheless, it is impossible to deny the influence of selection bias due to the presence of departments at this hospital specializing in the treatment of diabetes. Although there was an intense focus on patients for whom a detailed clinical history was obtained in advance, who

had a family history not inconsistent with maternal inheritance as well as negative autoantibodies, juvenile onset, complications of deafness and other diagnostic characteristics, these were assumed not to be linked to a comparatively high mutation detection ratio. Thus far, A3243 G mutations[9], T14709C mutations[6], C3256T, and other mtDNA partial deletions[10] are reported to be causative in diabetes related to mitochondrial disease. The mutations detected in patients with diabetic complications in this study were as follows: A3243G mutation in 16/17 subjects and T14709C mutation in 1 subject. These mutations were the same as those reported for MIDD. In mitochondrial diabetes, it is estimated that mutant beta-cells initially display reduced stimulus-secretion coupling, followed by beta-cell loss [11]. Mitochondrial diabetes is also associated with decreased oxidative phosphorylation and fatty acid oxidation in insulin sensitive tissues[12]. Based on these findings, we first studied all the A3243G mutations in subjects for whom maternal inheritance was clear and clinical mitochondria diabetes was suspected. In addition, we initially did not select a highly invasive method for sample collection. We considered that blood sample collection rather than more invasive specimen collection was a reasonable as long as we informed the patient of the possibility of a low mutation detection rate when obtaining informed consent from the patient.

Mitochondrial disease could not be completely ruled out in five subjects in this study in which a mutation was not detected. First, even if the mtDNA contained a mutation, it is possible that the mutation analysis parameters were insufficient or that the specimen tissue was inadequate. Next, in the five subjects, there was no family history suggesting maternal inheritance, so the cause of the mitochondrial disease could have been a nuclear gene, but nuclear genes were not examined in this study. In a future study, we will widen the parameters of the mtDNA mutation analysis, adding a nuclear gene test, and varying the sample tissues so that the mutations can be detected.

Symptoms will likely manifest if the mtDNA mutation exceeds a particular threshold. Confirmation of a mtDNA mutation does not necessarily mean that the subject will develop symptoms, as reported previously[13]. For patients with mitochondrial disease, as well as their families, uncertainty (e.g., what will happen to them given their present symptoms, what type of symptoms will manifest, when will the symptoms manifest, how their children and family will be affected, and the fact that they do not know what lies ahead) is a source of great stress. People seeking genetic counseling should be given clear information about the disease as well as its uncertainties and must be supported during the process of receiving and understanding information about the uncertainties.

Five subjects received genetic counseling regarding symptoms before the onset of any disease. When the cause of familial mitochondrial disease is maternal inheritance based on mtDNA mutations, the mutation proportion differs in each tissue. As a result, whether or not mutations are detected in at-risk subjects who do not present with symptoms, it is difficult to predict the future onset of disease in these subjects or to make detailed predictions about their symptoms. In addition, even when it is possible to make predictions and assumptions about the disease, the fact that there are no preventive measures or basic methods of treatment is a problem for diagnosis before the onset of a serious illness. Among mitochondrial diseases, however, symptoms vary so that information regarding the clinical symptoms of those in the family who are sick may not be of use in the diagnosis of blood relatives. Many at-risk people who are free of symptoms request a presymptomatic examination of at-risk persons. Even after we have explained the details of inheriting a mitochondrial disease and the uncertainties inherent in the genetic testing, we carried out the genetic testing if the demand was particularly forceful. After the A3243G mutation was detected in subject No.23, she became pregnant and was diagnosed with diabetes, so the genetic information was useful for selecting an institution for childbirth. While presymptomatic diagnosis is not the best option for all subjects, this information can be useful in managing the medical records for women who are at risk of developing the disease during pregnancy.

While this does not involve presymptomatic diagnosis, sometimes the presence of a mtDNA mutation in the mother and siblings with no symptoms or very mild symptoms is ascertained theoretically by confirming the mutation in a proband. In this study, 42.0 % of those who came for treatment had a family member at risk based on their own diagnosis, and subjects who were diagnosed or for whom the presence of mutations was confirmed theo-

retically accounted for 30.8 % of these. Although there are many cases in which an unexpected diagnosis can bring about intense upset, providing client treatment information to a person assumed to have a mtDNA mutation and to persons at risk in the family is beneficial for the future medical care of that person. As a result, when a client undergoes genetic testing, the client should be told beforehand that it is possible to partially infer genetic information regarding a blood relative based on the test results for the client and sometimes that information should be shared with the family member at that time.

In this study, women accounted for approximately two-thirds of those who requested a consultation. In particular, women of childbearing age who were single, without children, and pregnant accounted for 25.8 % of the women. There were sex differences among those who came for consultation, and among the men the consultations involved their own diagnosis in all cases. Women, however, came regarding treatment for their family members rather than for themselves. There is a common misconception that mitochondrial disease is inherited maternally and it may be that this affected their motivation to come to the hospital as well as the details of the consultation. Actually, mtDNA contain about 5 to 10 times more mutations than nuclear DNA and 1 in 200 newborns has a mtDNA mutation, which accounts for 1/5 of all neonatal mutations[14]. As a result, even though there are mutations in the mtDNA, they are by no means restricted to those inherited from the mother. Some mitochondrial diseases are caused by mutations in the nuclear DNA. Carrying the mutations is not the fault of the individual and the individual should not feel guilty. Realistically, however some clients may feel a baseless guilt for their situation. Particular attention is required for women and even if a couple comes in for a consultation, they may need to be seen separately with the woman given priority when her partner is present.

Almost none of the consultations were concerned with estimating the rate of recurrence for the next child and an examination before giving birth. When heteroplasmy relates to the manifestation of symptoms in the mtDNA mutations that are inherited from the mother, it is difficult to make a diagnosis prior to giving birth and we believe that in these cases the consultation should concentrate on providing information about seeking a highly specialized medical institution for the birth. This is technically possible when there is an onset with homoplasmy, as is the case with mt8993 mutations even when inherited from the mother[15)16)], and when nuclear gene mutations, which have been reported more than 200 times[17)], are the cause. The need for genetic counseling in prenatal examinations and in the perinatal period will most like increase as the causes of mitochondrial disease are confirmed.

Conclusions

We reported on genetic counseling experience relating to mitochondrial disease carried out at this center from April 2004 to November 2015. Thirty-one adults with a mean age of 40 years came for counseling. Genetic test results were confirmed in 74.1 % (20/27) of the subjects. The mutation detection ratio in blood samples obtained from those with symptoms was 77.3 % (17/22 subjects). Of the 31 subjects who came for counseling, 13 (42.0 %) had a family member who was at risk based on the client's diagnosis. Eight (25.8 %) of the 31 subjects who came for counseling were women that were single, childless, or pregnant. We believe that it is extremely important to evaluate all of the symptoms to provide a detailed history relating to mitochondrial disease and to provide genetic counseling that is appropriate for confirming an exact diagnosis based on clinical information and to confirm the pathogenesis. In some cases, the pathogenesis was not identified, and even when it was identified, it was often difficult to provide insight as well as to follow the progress of the disease. As a result, we believe that it is extremely important to organize clear information about the disease and its uncertainties, to empathetically listen to the subjects, and to assist the patients with the process of adapting to their situation. Long-term follow up is indispensable, as is follow-up of new patients in the client's family and continuous involvement in these interactions.

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Conflicts of Interest: No potential COI to disclose.

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