【 CASE REPORT 】

Observation of p.R4810K, a Polymorphism of the Mysterin Gene, the Susceptibility Gene for Moyamoya Disease, in Two Female Japanese Diabetic Patients with Familial Partial Lipodystrophy 1

Masanori Iwanishi [1], Choka Azuma [1], Yuji Tezuka [1], Yukako Yamamoto [1], Jun Ito-Kobayashi [1],
Miki Washiyama [1], Toru Kusakabe [2] and Shingo Kikugawa [3]

Abstract:
Mysterin, which was recently shown to play an important role in maintaining cellular fat storage, has been identified to be the susceptibility gene for moyamoya disease (MMD). We encountered some female Japanese patients with partial lipodystrophy and MMD-like vascular lesions. This prompted us to examine whether mysterin variants may be present in these patients. We identified a mysterin variant, p.R4810K in two patients with MMD-like vascular lesions, who may fit the category of familial partial lipodystrophy (FPLD) 1. Our cases suggest the possibility that p.R4810K, in addition to atherogenic risk factors, might thus play a role in the development of atherosclerotic lesions in patients with FPLD1 and p.R4810K.

Key words: partial lipodystrophy, familial partial lipodystrophy 1, mysterin, mysterin mutation, p.R4810 K, moyamoya disease

Introduction

Moyamoya disease (MMD) is a rare chronic cerebrovascular disease that is characterized by bilateral stenosis/occlusion at the terminal portion of the internal carotid artery with prominent arterial collateral circulation, which resembles a "Puff of Smoke" which is called moyamoya in Japanese (1, 2). Its incidence peaks at approximately 5 years of age and then there is another peak in the mid-40s. There are nearly twice as many female patients as male patients (3). The initial manifestation is cerebral ischemic symptoms in children and cerebral ischemic and intracranial hemorrhage symptoms in adults. MMD occurs at a higher prevalence in East Asian countries, including Japan, Korea, and China, than in other countries worldwide. The annual incidence of MMD is estimated to be 0.35-0.54 per 100,000 person-years in Japan, which is approximately one tenth of that in Europe. The mysterin gene (moyamoya steno-occlusive

disease-associated AAA+ and RING finger protein), also known as the ring finger protein (RNF) 213 gene, has been identified to be the susceptibility gene of MMD (1, 2). The prevalence of the heterogenous missense variant, p.R4810K was found to be relatively high among East Asians. This variant is transmitted in an autosomal dominant manner. P.R 4810K is found at rates of >90% in Japanese patients with MMD, and 2-3% of the Japanese population are carriers of this variant (4). Based on the prevalence of MMD and rate of carriers of this variant, one out of 150 carriers will develop MMD. This variant is found in approximately 20% of patients without MMD and with intracranial major artery stenosis/occlusion (ICASO) (5). Morimoto et al. showed that this variant in patients without MMD correlated with coronary artery disease in the Japanese population (6). These findings indicate that this variant is an important factor for the pathogenesis of atherosclerosis in patients without MMD.

Mysterin was recently shown to play an important role in

Table 1.   Basal Characteristics of Patients with Partial Lipodystrophy.

Patient	Age (years)	DM age of onset (years)	BMI (kg/m^2)	HbA1c (%)	Plasma glucose (mg/dL)	Plasma insulin (μU/mL)	Serum cholesterol (mg/dL)	Serum triglycerides (mg/dL)	Serum HDL cholesterol (mg/dL)	AST (u/L)	ALT (u/L)	PLTS (10^4/μL)	Serum leptin (ng/mL)	Serum adiponectin (μg/mL)
1	48	35	30.0	6.9	ND	ND	211	755	34	29	41	29.6	36.1	7.1
2	69	60	25.5	6.6	98	7.9	173	157	51	18	27	38.2	ND	ND

AST: aspartate transaminase ALT: alanine transaminase, PLTS: platelets ND: not determined

Table 2.   Clinical Characteristics of Patients with Partial Lipodystrophy.

Patient	Clinical lipoatrophy	Fat deposition	Family history	Visceral fat area (cm^2)	DR	Albuminuria	eGFR (mL/min/1.73m^2)	Clinical observations & therapy
1	calves, thighs, buttocks, forearms	Trunk	mother	140.4	MI	Macro	8.8	Cerebral infarction, HT, Bilateral severe narrowing at the terminal portion of the internal carotid artery Chronic renal failure INS, TZDs
2	calves, thighs, buttocks, forearms	Trunk	mother, brother, sister	126.0	(-)	N	70.6	Cerebral infarction, HT, IHD Occlusion of the left anterior cerebral artery and left middle cerebral arterial narrowing of the left internal carotid artery SU, TZDs

IHD: ischemic heart disease, INS: insulin, TZDs: thiazolidinediones, SU: sulfonyl urea, HT: hypertension, DR: diabetic retinopathy, MI: mild non-proliferative diabetic retinopathy, N: normal albuminuria, Macro: macroalbuminuria

maintaining fat storage in cytoplasmic lipid droplets (LDs) through the specific elimination of adipose triglyceride lipase (ATGL) (7) and some mysterin mutations impaired its fat-stabilizing effects (7). We recently reported the clinical characteristics of 15 female Japanese patients with partial lipodystrophy (PL) in the outpatient clinic at Kusatsu General Hospital, who were diagnosed based on dual energy X-ray absorptiometry (DEXA), magnetic resonance imaging (MRI) findings, and the clinical characteristics (8). We encountered some female Japanese diabetic patients with PL and MMD-like vascular lesions (8-10). This prompted us to examine whether mysterin mutations may be present in these patients.

Case Reports

We identified 15 female Japanese patients with PL in the outpatient clinic at Kusatsu General Hospital (8). We performed whole exome sequencing on 8 out of the 15 patients with PL. Three patients with PL had MMD-like vascular lesions based on the findings of magnetic resonance angiography (MRA) studies. Five patients with PL did not have MRA studies for the evaluation of atherosclerosis because of several reasons. We confirmed the p.R4810K variant in mysterin in two patients (Patient 1 and 2) using direct sequencing. The clinical data from Patients 1 and 2 have been reported previously (8-10). The previous report showed that Patient 1 and Patient 2 might fit the category of familial partial lipodystrophy (FPLD)1 (8, 10). Their basal and clinical characteristics are shown in Tables 1 and 2, respectively.

The present study, including whole exome sequencing, was approved by the Ethics Committee of Kusatsu General Hospital. All patients agreed to participate in the present study and provided their written informed consent. We also obtained written informed consent from the patients who underwent genetic analyses.

Case 1

Patient 1 was a 48-year-old woman. At 35 years of age, she was diagnosed with diabetes by a family doctor based on the presence of high levels of HbA1c and postprandial glucose. At 40 years of age, she was transferred to the hospital emergency room due to convulsions and a disturbance of consciousness. Her brain MRI (Fig. 2E) and MRA (Fig. 2F) findings showed severe narrowing at the end of the internal carotid artery on both sides with an old cerebral infarction in the right anterior lobe. The narrowing in the proximal regions of the anterior and middle cerebral arteries on both sides is indicated by black arrows. When the patient was 43 years old, superficial temporal artery-middle cerebral artery (STA-MCA) anastomosis was subsequently performed. The patient was admitted to our hospital for diabetes care, when she was 48 years old. At that time, her height was 152 cm and she weighed 64 kg, which indicated a body mass index (BMI) of 27.2 kg/m^2. We assessed the body fat distribution in Patient 1 at the age of 48 years using DEXA and MRI studies, as shown in Fig. 1B, Fig. 2, and Table 3. A control group (Control 1) consisting of 41 healthy female volunteers aged between 50 and 59 years was recruited from the employees of Tanita. Another control group (Control 2)
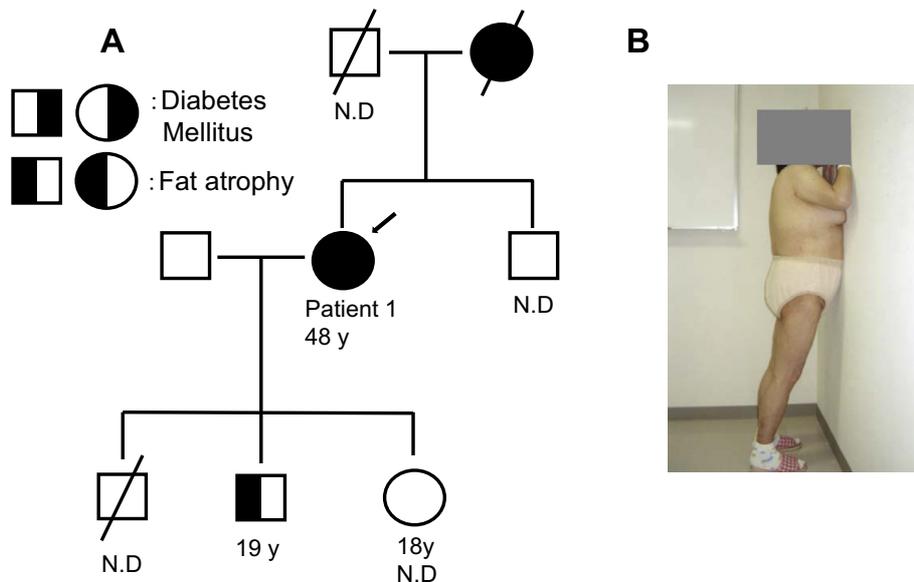
Figure 1. A: We present the family pedigree of Patient 1. The proband is denoted by an arrow. We had clinical data, including fat distribution on the patient's son. B: The phenotypic features of Patient 1 are shown. She had the loss of subcutaneous fat deposits in the forearms, lower limbs, and buttocks. She had excess fat deposition around the face, neck, and trunk, although that around the face and neck has been covered with gray paper for privacy reasons. Figure 1A and 1B were presented in our previous report (9).

with 139 healthy female volunteers aged between 29 and 80 years was also recruited from the employees of Tanita. The mean BMI of Control groups 1 and 2 were 23.5 and 25.4 kg/m², respectively. She had subcutaneous fat loss in her lower limbs, accompanied by excess subcutaneous fat in the trunk, compared with Control groups 1 and 2. We show the family pedigree of Patient 1 in Fig. 1A. While the patient's mother had a similar distribution of fat atrophy, according to the interview, computed tomography images of various regions of the patient's son in a previous report showed a similar distribution of fat atrophy (9). Thus, we considered an autosomal dominant pattern of inheritance. The patient's condition was clinically diagnosed as FPLD. The patient's mother was diagnosed with diabetes at 31 years of age and died of a cerebral infarction at 38 years of age. We could not perform a genetic analysis on any other family members.

Case 2

Patient 2 was a 69-year-old woman. At 60 years of age, she was diagnosed with diabetes by a diabetologist based on high levels of HbA1c. The body fat distribution was assessed in Patient 2 at 69 years of age using DEXA and MRI studies. As shown in Fig. 3B, Fig. 4, and Table 3, she had subcutaneous fat loss in her lower limbs, accompanied by excess subcutaneous fat in the trunk. According to the patient's interview, we show the family tree of Patient 2 in Fig. 3A. The patient's mother and sister had diabetes with a similar distribution of fat atrophy, according to the interview, indicating an autosomal dominant pattern of inheritance. We diagnosed her with FPLD based on the clinical characteris-

tics. We could not perform either genetic analyses or obtain any clinical data about any other family members. She had right hemiparesis and aphasia at 65 years of age and was diagnosed with cerebral infarction. As shown in Fig. 4E, her brain MRI at 69 years of age revealed an old cerebral infarction in the area of the left frontal lobe and temporal lobe, to which blood is supplied by the left middle cerebral artery. As shown in Fig. 4F, MRA showed a narrowing at the left internal carotid artery, occlusion of the left anterior artery, and a marked narrowing in the horizontal region of the left middle cerebral artery with occlusion in the distal region of the left middle cerebral artery. Coronary angiography showed 25% narrowing of #5, 99% narrowing of #6, and 75% narrowing of #7. Thus, this patient was diagnosed with severe atherosclerosis.

Genetic analyses

We performed whole-exome sequencing to examine whether or not the patients had mutations in candidate genes known to be associated with FPLD, including LMNA, PPARG, PLIN1, CIDEC, LIPE, ADRA2, and AKT2 (11). We found that Patient 1 and Patient 2 had neither mutations in these candidate genes known to be associated with FPLD nor any novel candidate causal genes, except for the mysterin variant, p.R4810K. Whole exome sequencing detected p.R4810K in both patients (Patient 1, 2). By direct sequencing, we confirmed p.R4810K in Patient 1 and Patient 2, as shown in Fig. 5.
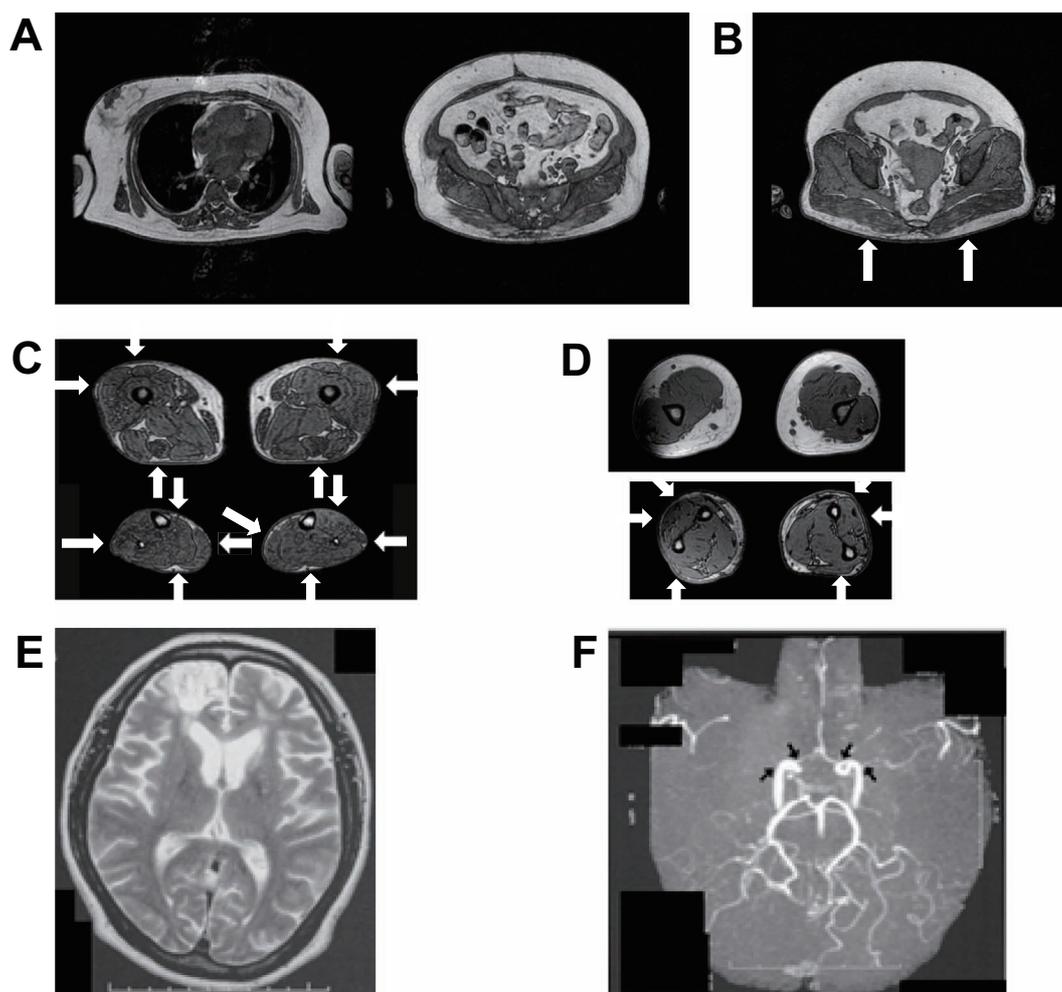
Figure 2. The figure shows magnetic resonance imaging (MRI) results for Patient 1. A: Thoracic MRI taken at the level of the seventh thoracic vertebrae (left panel) and abdominal MRI taken at the umbilical level (right panel) showed the preservation of subcutaneous fat in the thoracic and abdominal regions. B: A T1-weighted MRI taken at the level of gluteal fat indicated the prominent loss of gluteal subcutaneous fat, indicated by arrows. C: MRI taken at the level of the thigh (upper panel) and calf (lower panel) revealed the nearly complete absence of subcutaneous fat, particularly in the antero-lateral and posterior thigh regions and the entire circumference of the calf regions, indicated by arrows. D: MRI taken at the level of the arms (upper panel) and forearms (lower panel) revealed the marked loss of subcutaneous fat, particularly in the antero-lateral and posterior forearms, indicated by arrows, and the preservation of fat in the arms. Brain MRI, which had been performed when Patient 1 was transferred to the emergency room for convulsions at 40 years of age, showed an old infarction in the right frontal lobe (Fig. 2E), while brain magnetic resonance angiography (MRA) showed severe narrowing at the terminal portions of both sides of the internal carotid artery (Fig. 2F). Narrowing in the proximal regions of both sides of the anterior cerebral and middle cerebral arteries is indicated by black arrows.

Discussion

In evaluations of DEXA and MRI findings, Patients 1 and 2 had subcutaneous fat loss in their lower limbs, accompanied by excess subcutaneous fat in the trunk. They also noted fat loss during childhood or adolescence and likely had the autosomal dominant inheritance of lipodystrophy according to interview findings. Patients 1 and 2 had neither mutations in these candidate genes known to be associated with FPLD nor novel likely candidate causal genes, except for the mysterin variant. FPLD1 is generally autosomal dominant and it is characterized by fat loss in the lower limbs with abdominal fat accumulation, a high rate of positivity for a family history, unknown genetic cause, and metabolic disturbances (11-13). Therefore, based on these data, we speculated that Patients 1 and 2 may fit the category of FPLD1.

The mysterin protein shows a diffuse distribution in the cytosol and is targeted to LDs in most cells (7). It is com-

Table 3. Body Composition Assessed by DEXA in Patients with Partial Lipodystrophy.

Patient	Age (years)	BMI (kg/m²)	Total fat (%)	Upper limbs %fat (%)	Trunk %fat (%)	Lower limbs %fat (%)	Total fat mass (kg)	Upper limbs fat mass (kg)	Trunk fat mass (kg)	Lower limbs fat mass (kg)	Total lean mass (kg)	Upper limbs lean mass (kg)	Trunk lean mass (kg)	Lower limbs lean mass (kg)	FMR	Modified FMR
1	48	30.0	32.1	43.8	37.9	15.1	21.3	3.1	15.0	2.3	42.4	3.3	23.9	12.0	2.5	6.5
2	69	25.5	36.8	52.5	40.1	26.1	22.1	4.0	13.5	3.9	36.5	3.5	20.0	10.1	1.5	3.5
Control 1 (n=41)	55.2 (3.1)	23.5 (2.9)	31.4 (6.5)	29.6 (7.8)	31.2 (7.6)	32.5 (6.2)	17.3 (5.3)	1.6 (0.3)	8.0 (2.9)	6.5 (0.9)	34.6 (3.0)	3.4 (0.3)	16.3 (1.7)	12.3 (0.7)		
Control 2 (n=139)	52.8 (12.7)	25.4 (3.3)	34.9 (6.1)	32.4 (7.2)	34.4 (7.5)	36.1 (5.7)	20.9 (5.7)	1.8 (0.6)	9.5 (3.2)	8.0 (2.3)	35.7 (3.3)	3.4 (0.5)	16.8 (1.7)	13.1 (1.8)	1.0 (0.2)	1.2 (0.4)

Control 1 and Control 2 are in the bottom 2 sections. The figures in parentheses are SD. Normal values in Control 1 were obtained from 41 healthy women between the ages of 50 and 59 years. Normal values in Control 2 were obtained from 139 healthy women between the ages of 29 and 80 years.
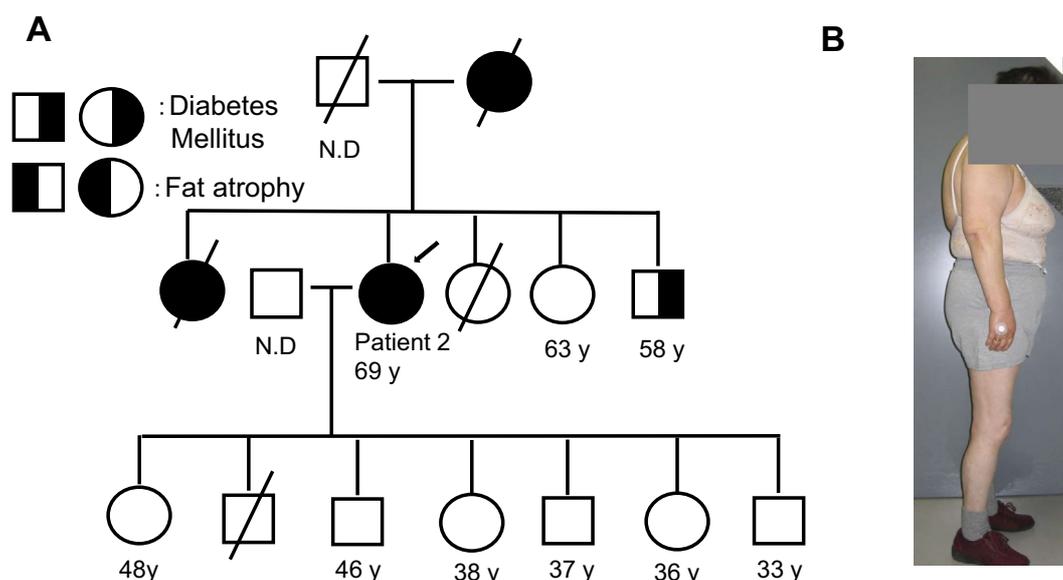
Figure 3.   A: We present the family pedigree of Patient 2, according to the patient's interview. The proband is denoted by an arrow. B: The phenotypic features of Patient 2 are shown. She had the loss of subcutaneous fat deposits in the forearms, lower limbs, and buttocks. She had excess fat deposition around the face, neck, and trunk, although that around the face and neck has been covered with gray paper for privacy. Figure 3B were presented in our previous report (10).

posed of 5,207 amino acids and has an estimated molecular size of 591 kDa. It has two AAA+ (ATPases associated with diverse cellular activities) modules and a single RING finger ubiquitin ligase domain (7, 14). This protein exhibits both AAA+ ATPase and ubiquitin ligase activities; however, their functions in cells remain unknown. The lipolysis of triglycerides (TGs) is mediated in a stepwise manner by three lipid droplet (LD) surface lipases: adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoacylglycerol lipase. ATGL is the most influential and rate-limiting lipase (15, 16). A recent study reported that mysterin plays an important role in maintaining fat storage in cytoplasmic LDs, presumably reducing ATGL at LDs by preventing its influx into LDs (7). Therefore, we speculated that dysfunctions in mysterin due to mutations may decrease TGs in LDs by increasing ATGL at LDs through the acceleration of its influx into LDs, thereby impairing the func-

tions of various cells, including endothelial cells, smooth muscle cells, and adipocytes.

Occlusive lesions in the vessels of MMD are caused by the excessive proliferation of smooth muscle cells, endothelial injury, and intimal hyperplasia (1). Insufficient angiogenesis was observed in transgenic mice, in which the mouse homolog of human p.R4810K was overexpressed specifically in endothelial cells, and mysterin knockout mice (17, 18). Therefore, we speculated that p.R4810K, may lead to stenosis/occlusion of the terminal portion of the intracranial internal carotid artery through these vascular disorders. The heterozygous variant p.R4810K in mysterin is found at a rate of >90% in Japanese patients with MMD (1). However, due to low genetic penetrance, carriers of this variant do not always develop MMD (1, 4); therefore, additional environmental and/or genetic factors are considered to be important for the development of MMD in
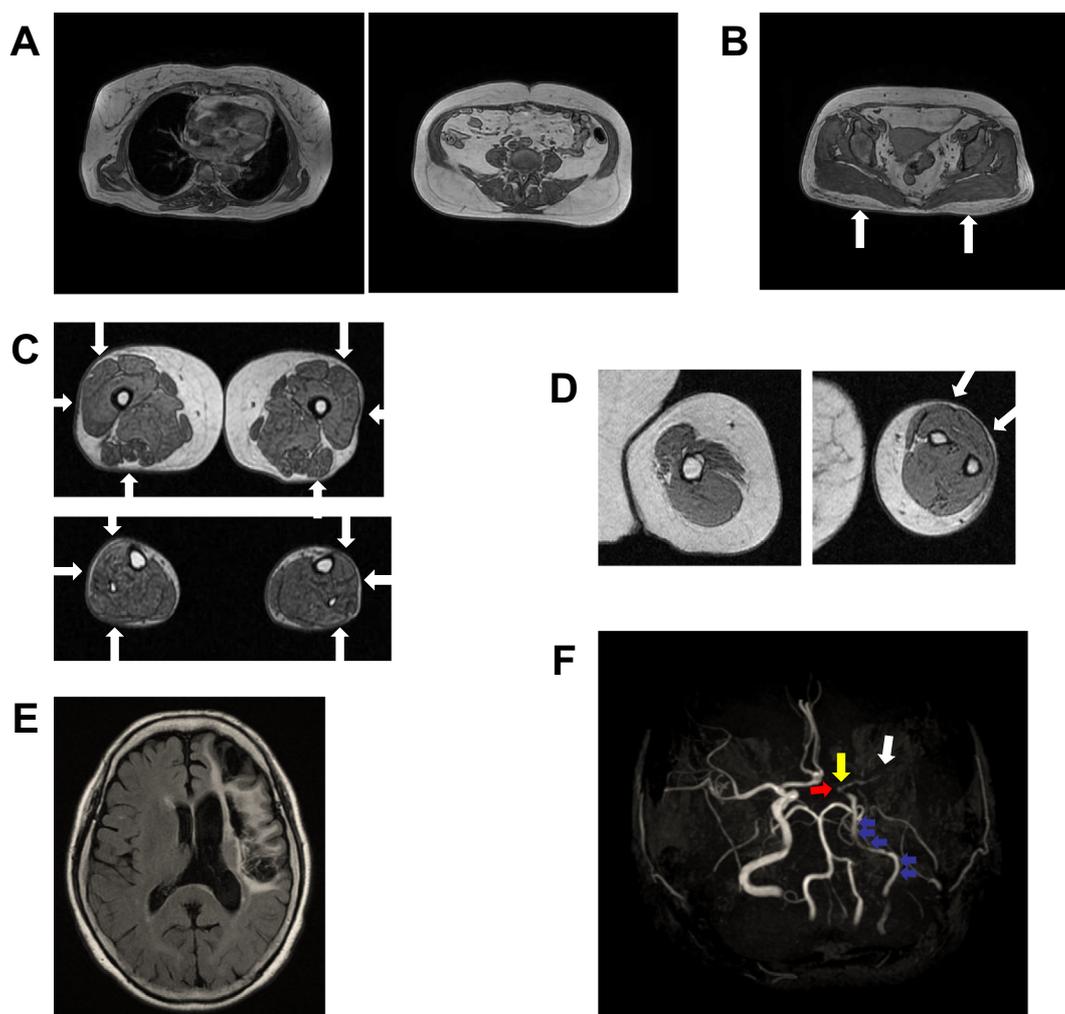
Figure 4. A-E were presented in our previous report (10). Patient 2 was a 69-year-old woman with a BMI of 25.5 kg/m². A: Thoracic MRI at the level of the seventh thoracic vertebrae (left panel) and abdominal MRI at the umbilical level (right panel) are shown. Thoracic and abdominal MRI revealed the preservation of subcutaneous fat in the thoracic and abdominal regions of Patient 2. B: T1-weighted MRI at the middle level of the gluteus are shown. MRI at the middle level of the gluteus in the proband revealed a decreased amount of gluteal subcutaneous fat, indicated by arrows. C: MRI at the middle level of the thigh (upper panel) and calf (lower panel) in Patient 2 are shown. The patient had the marked loss of subcutaneous fat, particularly in the antero-lateral and posterior thigh regions, indicated by arrows, although subcutaneous fat in the internal thigh region remained. She had the near-complete loss of subcutaneous fat in the antero-lateral and posterior calf regions, indicated by arrows; however, very little subcutaneous fat in the internal calf region remained. D: MRI at the middle level of the left arm (left panel) and forearm (right panel) are shown. Axial MRI at the level of the arm revealed the preservation of subcutaneous fat. Axial MRI at the level of the forearm revealed the almost complete absence of subcutaneous fat in the antero-lateral forearm, indicated by arrows; however, the preservation of subcutaneous fat in the internal forearm was observed. As shown in Fig. 4E, brain MRI at the age of 69 years showed old cerebral infarction in the area of the left frontal lobe and temporal lobe, to which blood is supplied by the left middle cerebral artery. As shown in Fig. 4F, MRA demonstrated a narrowing at the left internal carotid artery (blue arrow), occlusion of the left anterior artery (red arrow), and a marked narrowing in the horizontal region of the left middle cerebral artery (yellow arrow) with occlusion in the distal region of the left middle cerebral artery (white arrow).

carriers of p.R4810K. Therefore, the missense variant, p.R 4810K in the mysterin gene is considered to be a susceptibility gene, not a causative gene.

Previous studies reported that patients with FPLD1 may be predisposed to the development of severe atherosclerosis (8, 10, 12, 13). Atherosclerosis has been considered to be caused by the accumulation of atherogenic risk factors, such as diabetes, dyslipidemia, hypertension, and low levels of
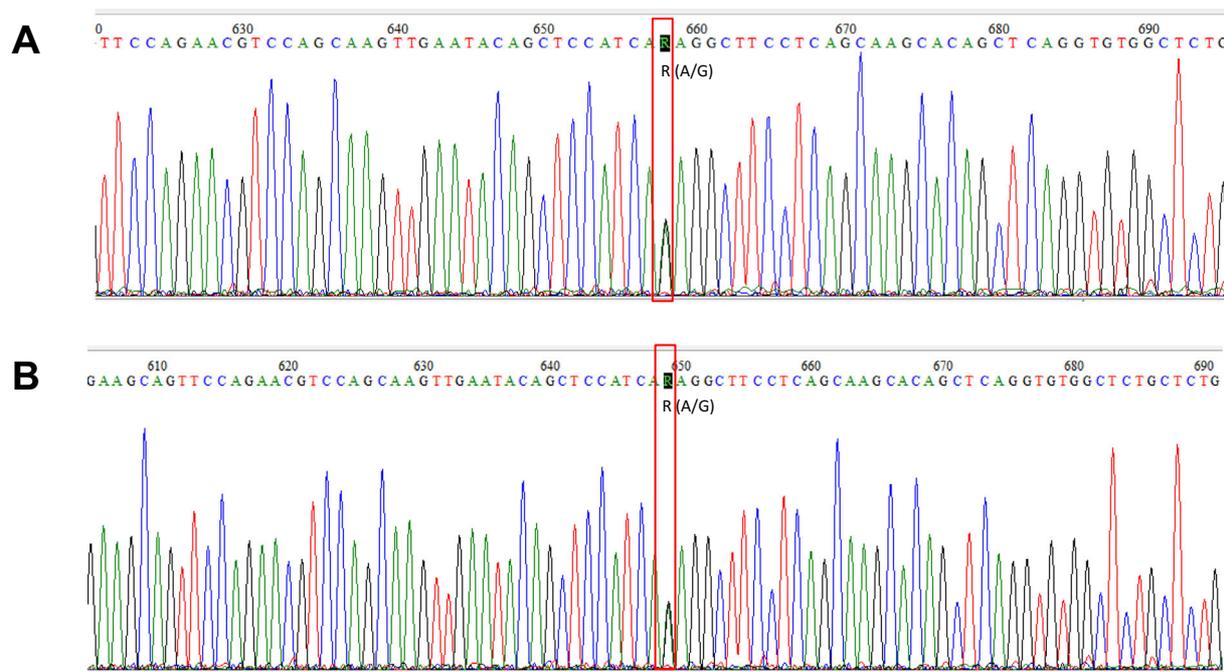
Figure 5. Reversed sequencing chromatograms showed the c.14576 G>A heterozygous mutation (p.R4810K) in the mysterin gene. A: Patient 1, B: Patient 2

serum adiponectin in patients with FPLD1. These risk factors are often caused by insulin resistance due to lipodystrophy. As Patients 1 and 2 had atherogenic risk factors, such as diabetes, dyslipidemia, and hypertension, it was conceivable that these factors contribute to MMD-like vascular lesions. Furthermore, since Patients 1 and 2 had the mysterin mutation p.R4810K, we speculated that these vascular disorders caused by p.4810K might have a modified effect on the atherosclerotic lesions observed in patients with FPLD1.

When examining vascular lesions at the terminal portions of the internal carotid arteries in patients with MMD, asymptomatic MMD carriers, and carriers in the general population, carriers of this mutation showed various clinical stages: slight to marked stenosis or occlusion at the terminal portions of the bilateral internal carotid arteries, slight to marked stenosis or occlusion at the terminal portion of the unilateral internal carotid artery, and the absence of abnormal findings (1, 19, 20). Based on the follow-up findings of vascular lesions in intra-family MMD carriers, this variant may contribute to various courses: the rapid progression of lesions, slow progression of lesions, and the absence of abnormal findings. In Patient 1, diabetes mellitus was diagnosed at 35 years of age, and cerebral infarction occurred at 40 years of age. The patient's mother was diagnosed with diabetes mellitus at 31 years of age, and she died of a cerebral infarction at 38 years of age. DEXA in Patient 1 showed a marked decrease in the lower limb fat volume. Stenosis of the bilateral internal carotid arteries was marked, and the deterioration of arteriosclerotic lesions at other intracranial arteries was observed during the subsequent course. By assuming that the onset of cerebral infarction is related to the accumulated risk of arteriosclerosis, the timing

was too early, and another factor, such as this variant, may thus have been involved. Patient 1 corresponded to a patient with the rapid progression of a lesion. In Patient 2, diabetes mellitus was diagnosed at 60 years of age, and cerebral infarction occurred at 65 years of age. On DEXA, the decrease observed in the lower limb fat volume was less marked than that in Patient 1 due to residual fat in the medial femoral region, as indicated on MRI. Cerebral infarction developed at an advanced age; Patient 2 corresponded to a patient with a slow progression of the lesion. These results suggest that both clinical courses: rapid and slow progression, may be possible if this mutation influences arteriosclerotic lesions. This is similar to clinical course of MMD.

Quasi-moyamoya disease refers to the presence of MMD-like vascular lesions accompanied by an underlying disease, such as Down syndrome, atherosclerosis. Morimoto et al. reported that p.R4810K was significantly associated with quasi-moyamoya disease (21). Chong et al. identified p.R 4810K in a Down syndrome patient who had the early onset of MMD-like vascular lesions (22). They speculated that Down syndrome and this variant may contribute to the development of vascular lesions. Thus, there is the possibility that PL might be one of underlying diseases associated with quasi-moyamoya disease and p.R4810K.

The perilipin 1 gene is a causative gene for FPLD 4 (11). Perilipin 1 protein mutations, which are caused by a few heterogenous mutations in the perilipin 1 gene, lead to lipodystrophy, a failure to inhibit lipolysis in ATGL, and decreasing TGs in LDs under basal conditions. A recent study reported that the anti-perilipin antibody is a causal agent in acquired generalized lipodystrophy due to autoimmune disease (23). Thus, these LD surface lipases are important

molecules for the pathogenesis of lipodystrophy. Significant fat loss has been observed in HeLa cells in which Caucasian cysteine/histidine mutations in the mysterin gene were over-expressed by transfection (7). Therefore, based on the function of the mysterin protein, there is the possibility that dysfunctions in mysterin due to mutations may decrease TGs in the LDs of adipocytes by increasing ATGL at LDs through the acceleration of its influx into LDs, and thus resulting in lipodystrophy. However, MMD patients with p.R4810K have never been previously reported to have lipodystrophy. In addition, significant fat loss was not observed in HeLa cells in which p.R4810K was overexpressed by transfection (7). Previous epidemiological studies did not link the carriers of this variant with any metabolic anomalies, such as diabetes and dyslipidemia (4). Thus, it remains unknown as to whether this variant is associated with lipodystrophy. Therefore, further studies are needed to clarify this association.

On evaluating the pathological roles of p.R4810K on atherosclerosis in patients with PL, we should take the following things into consideration. We did not obtain any direct evidence to show that this variant contributes to atherosclerosis in Japanese female patients with FPLD1 and this variant. Furthermore, only a small number of subjects were examined. This variant was found in approximately 20% of patients without MMD and with intracranial major artery stenosis/occlusion (ICASO) (5). Therefore, this variant may have been coincidently detected in patients with PL and IC-ASO. In addition, from 2-3% of the Japanese population are carriers of this variant. Since the majority of carriers with this variant in the general population are asymptomatic, they may be at a low risk of stenosis/occlusion at the terminal portions of the internal carotid arteries (4); however, the prognosis of asymptomatic patients remains unclear (24). Therefore, this variant might only slightly contribute to atherosclerosis in patients with FPLD1.

We performed whole exome sequencing in 8 out of the 15 patients with PL. Two of 3 FPLD1 patients with MMD-like vascular lesions had p.R4810K polymorphism in mysterin gene and the other did not have it. This is the summary of the present study.

In conclusion, this is the first report to identify p.R4810K in two female Japanese diabetic patients with FPLD1 and occlusive or stenotic lesions at the terminal portions of the internal carotid arteries. Atherosclerosis in patients with FPLD1 has been considered to be caused by the accumulation of atherogenic risk factors. In addition, since p.R4810K is regarded as an important factor for the development of vascular disorders, the present results suggest the possibility that the vascular disorder caused by p.R4810K might have a modified effect on atherosclerosis and be an important target for therapeutic strategies in patients with FPLD1 and p.R 4810K. However, we examined only 3 patients who had both data sets of brain MRA and the mysterin gene sequence and we could neither confirm nor deny the contribution of p.R4810K polymorphism to the pathogenesis of MMD-like vascular lesions in this study. Further studies are required to confirm this relationship with p.R4810K in a large number of female Japanese patients with FPLD1.

References

1. Liu W, Morito D, Takashima S, et al. Identification of RNF 213 as a susceptibility gene for moyamoya disease and its possible role in vascular development. PLOS one **6**: e22542, 2011.
2. Kamada F, Aoki Y, Narisawa A, et al. A genome-wide association study identifies RNF 213 as the first Moyamoya didease gene. J Hum Genet **56**: 34-40, 2011.
3. Scott RM, Smith ER. Moyamoya disease and moyamoya syndrome. N Engl J Med **360**: 1226-1237, 2009.
4. Cao Y, Kobayashi H, Morimoto K, et al. Frequency of RNF 213 p.R4810K, a susceptibility variant for moyamoya disease, and health characteristics of carriers in the Japanese population. Environ Health Prev Med **21**: 387-390, 2016.
5. Miyawaki S, Imai H, Shimizu M. Genetic variant RNF 213 c. 14576G>A in various phenotypes of intracranial major artery stenosis/occlusion. Stroke **44**: 2894-2897, 2013.
6. Morimoto T, Mineharu Y, Ono K, et al. Significant association of RNF 213 p.R4810K, a moyamoya susceptibility variant, with coronary artery disease. PLoS one **12**: e0175649, 2017.
7. Sugihara M, Morito D, Ainuki S, et al. The AAA+ATPase/ubiquitin ligase mysterin stabilizes cytoplasmic lipid droplets. J. Cell Biol **218**: 949-960, 2019.
8. Iwanishi M, Kusakabe T, Azuma C, et al. Clinical characteristics of 15 female Japanese patients with partial lipodystrophy identified in a diabetic outpatient clinic. Diabetes Updates. Forthcoming.
9. Iwanishi M, Ebihara K, Kusakabe T, et al. Premature atherosclerosis in a Japanese diabetic patient with atypical familial partial lipodystrophy and hypertriglyceridemia. Intern Med **51**: 2573-2579, 2012.
10. Iwanishi M, Ito-Kobayashi J, Washiyama M, et al. Clinical characteristics, phenotype of lipodystropy and genetic analysis of six Japanese female patients with partial lipodystrophy in a diabetic outpatient clinic. Intern Med **57**: 2301-2313, 2018.
11. Akinci B, Sahinoz M, Oral E. Lipodystrophy Syndrome: Presentation and Treatment Endotext [Internet]. MDText. com, Inc, South Dartmouth (MA), 2018: 2000.
12. Herbst KI, Purnell JQ, Tannock LR, et al. Köbberling type of familial partial lipodystrophy. Diabetes Care **26**: 1819-1824, 2003.
13. Guillin-Amarella C, Sanchez-Iglesias S, Castro-Pais A, et al. Type 1 familial partial lipodystrophy: understanding the Köbberling syndrome. Endocrine **54**: 411-421, 2016.
14. Morito D, Nishikawa K, Hoseki J, et al. Moyamoya disease-associated protein mysterin/RNF 213 is a novel AAA + ATPase, which dynamically changes its oligomeric state. Sci Rep **4**: 4442, 2014.
15. Zimmermann R, Strauss JG, Haemmerle G, et al. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science **306**: 1383-1386, 2004.
16. Zechner R, Madeo F, Kratky D. Cytosolic lipolysis and lipophagy: two sides of the same coin. Nat Rev Mol Cell Biol **18**: 671-684, 2017.
17. Kobayashi H, Matsuda Y, Hitomi T. Biochemical and functional characterization of RNF213 (Mysterin) R4810K, a susceptibility mutation of moyamoya disease, in angiogenesis *in vitro* and *in Vivo*. J Am Heart Assoc **4**: e002146, 2015.
18. Morimoto T, Enmi JI, Hattori Y. Dysregulation of RNF 213 promotes cerebral hypoperfusion. Sci Rep **8**: 3607, 2018.
19. Mineharu Y, Takagi Y, Takahashi J, et al. Rapid progression of unilateral moyamoya disease in a patient with a family history and

an RNF 213 risk variant. Cerebrovasc Dis **36**: 155-157, 2013.

20. Matsuda Y, Mineharu Y, Kimura M, et al. RNF213 p.R4810K variant and intracranial arterial stenosis or occlusion in relatives of patients with moyamoya disease. J Stroke Cerebrovasc Dis **26**: 1841-1847, 2017.

21. Morimoto T, Mineharu Y, Kobayashi H, et al. Significant association of the RNF213 p.R4810K polymorphism with quasi-moyamoya disease. J Stroke Cerebrovasc Dis **25**: 2632-2636, 2016.

22. Chong PF, Ogata R, Kobayashi H, et al. Early onset of moyamoya syndrome in a Down syndrome patient with the genetic variant RNF213 p.4810K. Brain Dev **37**: 822-824, 2015.

23. Corvillo F, Aparicio V, Lopez-Lera A. Autoantibodies against perilipin 1 as a cause of acquired generalized lipodystrophy. Front Immunol **9**: 2142, 2018.

24. Koizumi A, Kobayashi H, Hitomi T, et al. A new horizon of moyamoya disease and associated health risks explored through RNF 213. Environ Health Prev Med **21**: 55-70, 2016.