

[ORIGINAL ARTICLE]

Clinical Characteristics, Phenotype of Lipodystrophy and a Genetic Analysis of Six Diabetic Japanese Women with Familial Partial Lipodystrophy in a Diabetic Outpatient Clinic

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Abstract:

Objective Our aim was to examine the clinical characteristics and phenotype of lipodystrophy of six diabetic Japanese women with partial lipodystrophy (PL) who received a genetic analysis at a diabetic outpatient clinic.

Methods We screened for PL using dual energy X-ray absorptiometry (DEXA) and magnetic resonance imaging (MRI) among patients who had a reduced peripheral skinfold thickness at the diabetic outpatient clinic of Kusatsu General Hospital between August 2003 and August 2013. We performed a mutation analysis of candidate genes, including LMNA and PPARG, in two patients with PL and whole-exome sequencing in four patients with PL.

Results We identified 15 patients with PL and performed a genetic analysis in 6 of them. They had no mutations in candidate genes known to be associated with familial partial lipodystrophy (FPLD). They all had near-complete loss of subcutaneous fat, particularly in the antero-lateral and posterior thigh region and the calf region. As almost all patients were characterized by fat loss in the lower limbs with abdominal fat accumulation, a high rate of positivity for a family history, diabetes, and an unknown genetic cause, we suspected they might have FPLD1. Some patients have shown relatively severe insulin resistance, while others have shown insulin deficiency. Four and one had severe atherosclerosis and liver cirrhosis, probably due to nonalcoholic steatohepatitis, respectively.

Conclusion Almost all patients with PL identified in a diabetic outpatient clinic had subcutaneous fat loss in the lower limbs with excess truncal fat and might have had FPLD1.

Key words: diabetic outpatient clinic, partial lipodystrophy (PL), familial partial lipodystrophy (FPLD)

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Introduction

Lipodystrophy has been classified as either inherited or acquired with either general or partial loss of adipose tissue deposits (1-4). It is commonly associated with dyslipidemia, hepatic steatosis, and insulin-resistant diabetes. However, Strickland et al. reported a novel form of partial lipodystrophy of the limbs (PLL) that does not resemble entities that

are conventionally categorized into the two-by-two classifications of congenital versus acquired and generalized versus partial (5, 6). This lipodystrophy is characterized by symmetrical distal lipodystrophy of the limbs and severe insulin resistance and appears to be acquired, based on the lack of a family history and its onset in adulthood.

Familial partial lipodystrophy (FPLD) is classified as inherited partial lipodystrophy. It is a clinically heterogeneous group of autosomal dominant disorders characterized by the

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variable loss of subcutaneous adipose tissue in the extremities. FPLD is also classified into eight subtypes of FPLD1-7 and AKT2-linked lipodystrophy (1, 2). FPLD1, otherwise known as Kobberling-type, is usually autosomal-dominant, but its genetic cause is unknown. Patients with FPLD1 are characterized by fat loss in the lower limbs with abdominal fat accumulation and a high rate of positivity for a family history (7). FPLD2 is associated with several mutations in the genes encoding laminin A/C and is thought to be the most common subtype (1). Lipodystrophy syndromes are diagnosed by the loss or absence of subcutaneous fat in a partial or generalized fashion, their history, and their clinical phenotype, supplemented by genetic testing for certain forms (1-4).

Although the prevalence of PLL was recently reported to be 0.79% in a Turkish diabetes clinic (6), the prevalence and clinical characteristics of any lipodystrophy type in type 2 diabetes remain unknown. Therefore, in order to examine the phenotype of lipodystrophy and the clinical characteristics of partial lipodystrophy (PL), we screened for PL using dual energy X-ray absorptiometry (DEXA) and magnetic resonance imaging (MRI) among patients who had a reduced peripheral skinfold thickness at the diabetic outpatient clinic of Kusatsu General Hospital between August 2003 and August 2013.

We performed genetic analysis in 6 of 15 patients with PL identified in our diabetic outpatient clinic, including cases reported previously (8-10). We herein report the phenotype of lipodystrophy and clinical characteristics of these six patients.

Materials and Methods

Study subjects

We screened for PL among the diabetic outpatients at Kusatsu General Hospital between August 2003 and August 2013, who were suspected of having subcutaneous fat loss in some parts of the body based on visual and physical examinations. We examined this suspected fat loss using DEXA and diagnosed PL based on symmetrical near-complete loss or complete loss of subcutaneous fat in peripheral sites as evaluated by MRI. Patients with PL were categorized into subgroups of PL (inherited or acquired, FPLD1-7) based on clinical observations, such as the onset of lipodystrophy, their family history, their personal history, and genetic information.

A control group (Control 1) with 41 healthy female volunteers between 50 and 59 years of age was recruited from among the employees of Tanita. Another control group (Control 2) with 139 healthy female volunteers between 29 and 80 years of age was also recruited from among the employees of Tanita. The mean body mass index (BMI) in Control groups 1 and 2 was 23.5 kg/m² and 25.4 kg/m², respectively. We evaluated the body composition as determined by DEXA in PL patients using Control 1 and Control

2 with their different mean BMI values. This study, including whole-exome sequencing, was approved by the ethics committee of Kusatsu General Hospital. The patients agreed to participate in the study and provided their verbal informed consent.

Methods

Blood samples and biochemical analyses

Blood was collected after a 12-h overnight fast for the analysis of glucose, insulin, leptin, and adiponectin. Plasma glucose was measured by the glucose oxidase method, and hemoglobin A1c (HbA1c) was measured by high-performance liquid chromatography. The serum insulin level was measured using commercial radioimmunoassay kits (Shionogi, Osaka, Japan). The serum leptin level was determined by commercial radioimmunoassay kits (Linco Research, St. Charles, USA). The serum adiponectin level was determined by commercial radioimmunoassay kits (Otsuka assay, Osaka, Japan) (11). The serum leptin and adiponectin levels were measured before thiazolidinediones (TZDs) were administered to the patients.

Oral glucose tolerance test

A standard oral glucose tolerance test (OGTT) with 75 g of glucose was performed after a 12-h overnight fast. Venous blood was collected for the determination of glucose and insulin concentrations immediately before glucose administration and at 30-minute intervals thereafter for 120 minutes.

DEXA

Whole-body DEXA was performed with a multiple detector fan-beam Hologic QDR-4500W densitometer (Hologic, Marlborough, USA) in the PL group and with a multiple detector fan-beam GE Lunar DPXL densitometer (General Electric, Fairfield, USA) in the control groups. Data were obtained from the head, upper limbs, trunk, and lower limbs. Proportions of fat in individual regions as well as in the whole body were calculated as percentages of the body mass.

MRI

MRI was performed using a 3.0-Tesla imaging device (Sigma Horizon; General Electric). The upper and lower limbs were examined using contiguous axial 10-mm slices. Fat was easily identified on MRI because of its short T1 relaxation time and its relatively high signal intensity on images compared with other tissues, such as muscle.

Measurement of the visceral fat area

Subcutaneous and visceral fat areas were measured using a -150- to -50-Hounsfield unit area using a modified method of computed tomography (CT; Light Speed Plus-R; General Electric) by Tokunaga et al. at the umbilical level (12).

Sequence analyses

We obtained written informed consent for genetic analy-

Table 1. Basal Characteristics of the Patients with Partial Lipodystrophy (PL).

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 triglyceride (mg/dL)	Serum HDL cholesterol (mg/dL)	AST (u/L)	ALT (u/L)	PLTS (10 ⁹ /μL)	Serum leptin (ng/mL)	Serum adiponectin (μg/mL)
1	46	34	22.9	12.0	245	17.7	200	137	49	12	10	27.4	10.4	7.9
2	48	35	30.0	6.9	ND	ND	211	755	34	29	41	29.6	36.1	7.1
3	47	33	27.6	9.3	ND	ND	266	171	65	13	13	34.0	6.2	2.3
4	69	60	25.5	6.6	98	7.9	173	157	51	18	27	38.2	ND	ND
5	50	41	22.2	6.5	127	12.8	177	79	46	25	37	17.7	4.9	4.1
6	67	47	25.2	8.4	95	8.8	181	92	56	45	34	23.9	12.1	8.1
Patient	54.5 (10.6) (n=6)	41.7 (10.4) (n=6)	25.6 (2.9) (n=6)	8.3 (2.1) (n=6)	141.3 (70.7) (n=4)	11.8 (4.5) (n=4)	201.3 (34.9) (n=6)	231.8 (258.8) (n=6)	50.2 (10.3) (n=6)	23.7 (12.4) (n=6)	27.0 (12.9) (n=6)	28.5 (7.3) (n=6)	13.9 (12.7) (n=5)	5.9 (2.6) (n=5)

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

Results

Clinical characteristics of the patients with PL

On evaluating DEXA and MRI findings, we diagnosed 15 women with PL among the diabetic outpatients at Kusatsu General Hospital between August 2003 and August 2013 who were suspected of subcutaneous fat loss in some parts of the body based on visual and physical examinations. The basal characteristics of the six women are shown in Table 1, and their clinical characteristics are shown in Table 2. The mean age at the diagnosis of lipodystrophy was 54.5 years, and the mean age at the diagnosis of diabetes was 41.7 years. The mean BMI was 25.6 kg/m².

The mean HbA1c was 8.3%. DEXA study results are presented in Table 3. All patients had a decreased lower limbs fat mass compared with Control groups 1 and 2, which had mean BMI values of 23.5 kg/m² and 25.4 kg/m², respectively. Three PL patients (Patients 4, 5, and 6) had a lower limb lean mass less than mean-2 standard deviations (SD) of Control 1.

The locations of lipodystrophy and fat deposition on MRI are presented in Table 2. The results of MRI assessments of body fat distribution in the control subjects are shown in Fig. 1. The body fat distribution in Patients 1, 2, and 3 was described in a previous paper (8-10), while the values in Patients 4, 5, and 6 are shown in Figs. 2-4, respectively. Four patients (Patients 2, 3, 4, and 5) had loss of subcutaneous fat in the forearms, calves, thighs, and buttocks. Two patients (Patients 1 and 6) had loss of subcutaneous fat in the calves, thighs, and buttocks. As shown in Fig. 5A, all patients had near-complete loss of subcutaneous fat in the antero-lateral and posterior thigh region, although the amount of subcutaneous fat in the internal thigh region was preserved.

There were some differences in the amounts of fat in the inter- and intramuscular adipose tissues and bone marrow. Patient 1 had preserved fat in the inter- and intramuscular adipose tissues in the thigh region, whereas other patients exhibited near-complete loss. Patients 2 and 3 had near-complete loss in the bone marrow in the thigh region, but the other patients had preserved fat. As shown in Fig. 5B, Patient 1 had very low subcutaneous fat in the entire circumference of the calf, while Patients 2, 3, and 5 had near-complete loss of subcutaneous fat in the entire circumference of the calf. Patients 4 and 6 had near-complete loss of subcutaneous fat in the calf region, particularly in the antero-lateral and posterior calf region, although they had low subcutaneous fat in the internal calf region. Patient 1 had preserved fat in the inter- and intramuscular adipose tissues in the calf region, while other patients had near-complete loss. The fat in the bone marrow in the calf region of all patients was preserved. Patient 5 had near-complete loss of subcutaneous fat in the entire circumference of the calf, although they had very low subcutaneous fat in the

ses from the patients subjected to genetic analyses. Sequence analyses of LMNA, PPARG, AKT2, and Caveolin-1 were performed using methods reported previously (8-10), and whole-exome sequencing was performed for Patients 3, 5, and 8 as reported previously (13).

Table 2. Clinical Characteristics of the Patients with Partial Lipodystrophy (PL).

Patient	Age (years)	Gender	BMI (kg/m ²)	Clinical lipoatrophy	Fat deposition	DM (age of onset)	Family history	Visceral fat area (cm ²)	Leptin (ng/mL)	Adiponectin (µg/mL)	Clinical observation & therapy
1	46	Female	22.9	calves, thighs, buttocks	Trunk	34	mother, two sisters	83.3	10.4	7.9	Relatively severe insulin resistance INS, TZDs (remarkable efficacy)
2	48	Female	30.0	calves, thighs, buttocks, forearms	Trunk	35	mother	140.4	36.1	7.1	Cerebral infarction HT INS, TZDs
3	47	Female	27.6	calves, thighs, buttocks, forearms	Trunk	33	mother	119.0	6.2	2.3	Middle cerebral artery occlusion Follicular lymphoma IHD, INS, TZDs
4	69	Female	25.5	calves, thighs, buttocks, forearms	Trunk	60	mother, brother, sister	126.0	ND	ND	Cerebral infarction HT IHD Middle cerebral artery occlusion SU, TZDs
5	50	Female	22.2	calves, thighs, buttocks, forearms		41	mother	124.0	4.9	4.1	ASO, Severe insulin resistance HT GLP-1RA
6	67	Female	25.2	calves, thighs, buttocks	Trunk	47	mother, sister	160.2	12.1	8.1	Liver cirrhosis probably due to NASH gastric cancer HT SU, TZDs

IHD: ischemic heart disease, NASH: nonalcoholic steatohepatitis, ASO: arteriosclerosis obliterans, HT: hypertension, INS: insulin, TZDs: thiazolidinediones, SU: sulfonyl urea, GLP-1RA: GLP-1 receptor agonist, ND: not determined

antero-lateral thigh region.

As shown in Table 2, the subcutaneous fat loss in the gluteal region and forearms was also accompanied by fat loss in the lower limbs in four of the six patients (Patients 2, 3, 4, and 5). All patients had subcutaneous fat loss in the gluteal region, and almost all patients had excess fat in the trunk (Patients 1-4 and 6). Thus, based on MRI and DEXA findings, subcutaneous fat loss in the lower limbs with excess truncal fat was predominant in this set of female Japanese patients with PL.

As shown in Table 2, five patients were treated with TZDs. Two patients were treated with sulfonylurea, one was treated with glucagon-like peptide-1 (GLP-1) receptor agonists, and three were treated with insulin injection. Interviews revealed that all patients had noticed subcutaneous fat loss in their lower limbs during childhood and adolescence, and all had a positive family history of lipodystrophy (Table 2). No were found to have any exposure to causes of acquired PL. Therefore, we suspected that all patients had FPLD.

The visceral fat area at the umbilical level was ≥ 100 cm² in 5 patients. Four patients had hypertension (Patients 2, 4, 5, and 6), and four (Patients 2, 3, 4, and 5) had severe atherosclerosis (cerebral infarction in 2 cases, ischemic Heart Disease (IHD) in 2 cases, arteriosclerosis Obliterans (ASO) in 1 case, middle cerebral artery occlusion in 2 cases). Patient 6 did not drink alcohol regularly or have any causes of liver cirrhosis, including viral infection and autoimmune hepatitis. When we evaluated the accumulation of visceral fat by CT at the umbilical level, the area of visceral fat was 160.2 cm², indicating visceral obesity. As shown in Table 1, Although the values of Aspartate transaminase (AST), Alanine transaminase (ALT), and Platelets (PLTS) (Table 1) and the CT findings in Patient 6 did not suggest severe Nonalcoholic Fatty Liver Disease (NAFLD), peritoneoscopy revealed nodular formation on the surface of the enlarged left hepatic lobe, consistent with liver cirrhosis or pre-cirrhosis (Fig. 4E), when she underwent laparoscopic cholecystectomy for cholelithiasis at 67 years of age.

As shown in Table 4, we performed an OGTT to estimate the insulin secretion and insulin resistance in four of six patients. Three patients (Patients 1, 5, and 6) exhibited relatively severe insulin resistance, whereas Patient 4 had a similar level of insulin secretion and insulin resistance to the average patient with type 2 diabetes.

Fig. 2 shows a representative case with a mild form of PL. Patient 4 was a 69-year-old woman. Her height was 157.0 cm, and she weighed 63.0 kg with a BMI of 25.5 kg/m². The regional and whole-body adipose tissue distribution and body composition estimated by DEXA scans are shown in Table 4. Compared with normal subjects, the patient had markedly low levels of fat in her legs, with prominent accumulation of fat in the trunk. On DEXA, the fat mass of the lower limbs in this patient was 3.9 kg, whereas this value was 1.7 kg in Patient 1, 2.3 kg in Patient 2, and 2.4 kg in Patient 3, as we reported previously (8-10). Thus, Patient 4

Table 3. Body Composition as Determined by DEXA Scan in Patients with Partial Lipodystrophy (PL).

Patient	Age (years)	BMI (kg/m ²)	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)
1	46	22.9	33.1	44.6	40.4	12.3	20.2	2.7	15.0	1.7	38.8	3.3	21.5	10.9
2	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
3	47	27.6	28.2	28.5	36.1	12.1	18.2	1.8	13.3	2.4	44.1	3.8	22.9	14.7
4	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
5	50	22.2	25.2	29.1	26.1	22.0	13.7	1.6	7.9	3.5	38.9	3.5	21.8	10.4
6	67	25.2	31.1	40.2	35.6	21.6	17.4	2.5	10.6	3.4	36.9	3.6	18.7	10.8
Patient (n=6)	54.5 (10.6)	25.6 (2.9)	31.1 (4.0)	39.8 (9.4)	36.0 (5.3)	18.2 (5.8)	18.8 (3.1)	2.6 (0.9)	12.6 (2.8)	2.9 (0.9)	39.6 (3.0)	3.5 (0.2)	21.5 (1.9)	11.5 (1.7)
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)

The means±SD in Patient, Control 1 and Control 2 are in the bottom 3 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. DEXA: dual energy X-ray absorptiometry

had reduced fat loss in the lower limbs compared with these three patients. As shown in Fig. 2A, she lost subcutaneous fat deposits in the forearm, lower limbs, and buttocks, with prominent lower limb musculature. She had excess fat deposition around the face, neck, and trunk, although that around the face and neck is covered with gray paper in the figure for privacy. As shown in Fig. 2B, thoracic (left panel) and abdominal MRI (right panel) revealed the preservation of subcutaneous fat in the thoracic and abdominal regions. As shown in Fig. 2C, MRI scans at the level of gluteal fat in the proband demonstrated a decreased amount of gluteal subcutaneous fat compared with the subcutaneous fat in the abdominal wall, indicating relatively low fat loss in the buttocks. As shown in Fig. 2D, the patient had marked loss of subcutaneous fat, particularly in the antero-lateral and posterior thigh region (upper panel), although subcutaneous fat in the internal thigh region was evident. She had near-complete loss of subcutaneous fat in the antero-lateral and posterior calf regions, although very low subcutaneous fat in the internal calf region remained. As shown in Fig. 2E, axial MRI at the level of the arm (upper panel) indicated preservation of subcutaneous fat. Axial MRI at the level of the forearm (lower panel) revealed almost complete absence of subcutaneous fat in the antero-lateral forearm, although there was preserved subcutaneous fat in the internal forearm. At 60 years of age, she was diagnosed with diabetes by a diabetologist based on her high levels of HbA1c. She had no history of autoimmune or infectious diseases, nor did she have diabetic retinopathy. Her total urinary albumin level was 22.5 mg/day, which is consistent with no diabetic nephropathy. She noticed a loss of subcutaneous fat over her forearms, lower limbs, and buttocks at 14 years of age. The patient's mother, sister, and brother had diabetes with a similar distribution of fat atrophy, indicating an autosomal dominant

pattern of inheritance. We diagnosed her with FPLD based on her family history of lipodystrophy and the onset of subcutaneous fat loss in DEXA and MRI studies. As shown in Table 4, Patient 4 had similar OGTT results to those of the average type 2 diabetes patient. She had cerebral infarction in the area of the left frontal lobe and temporal lobe, to which blood is supplied by the left middle cerebral artery. Magnetic resonance angiography demonstrated narrowing at the left internal carotid artery and 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.

Sequence analyses

We examined the sequences of the entire coding region and exon-intron boundary regions of LMNA, PPARG, AKT 2, and Caveolin-1 in Patient 1 and LMNA, PPARG, and Caveolin-1 in Patient 2. We found no mutations in these genes in these patients (9, 10). Whole-exome sequencing in Patients 3, 4, 5, and 6 showed no mutations in candidate genes known to be associated with FPLD.

Discussion

On evaluating DEXA and MRI findings, we diagnosed 15 women with PL among the diabetic outpatients at Kusatsu General Hospital between August 2003 and August 2013 who were suspected of subcutaneous fat loss in some parts of the body based on visual and physical examinations. Six patients who underwent a genetic analysis had near-complete loss of subcutaneous fat, particularly in the antero-lateral and posterior thigh region and the calf region, as

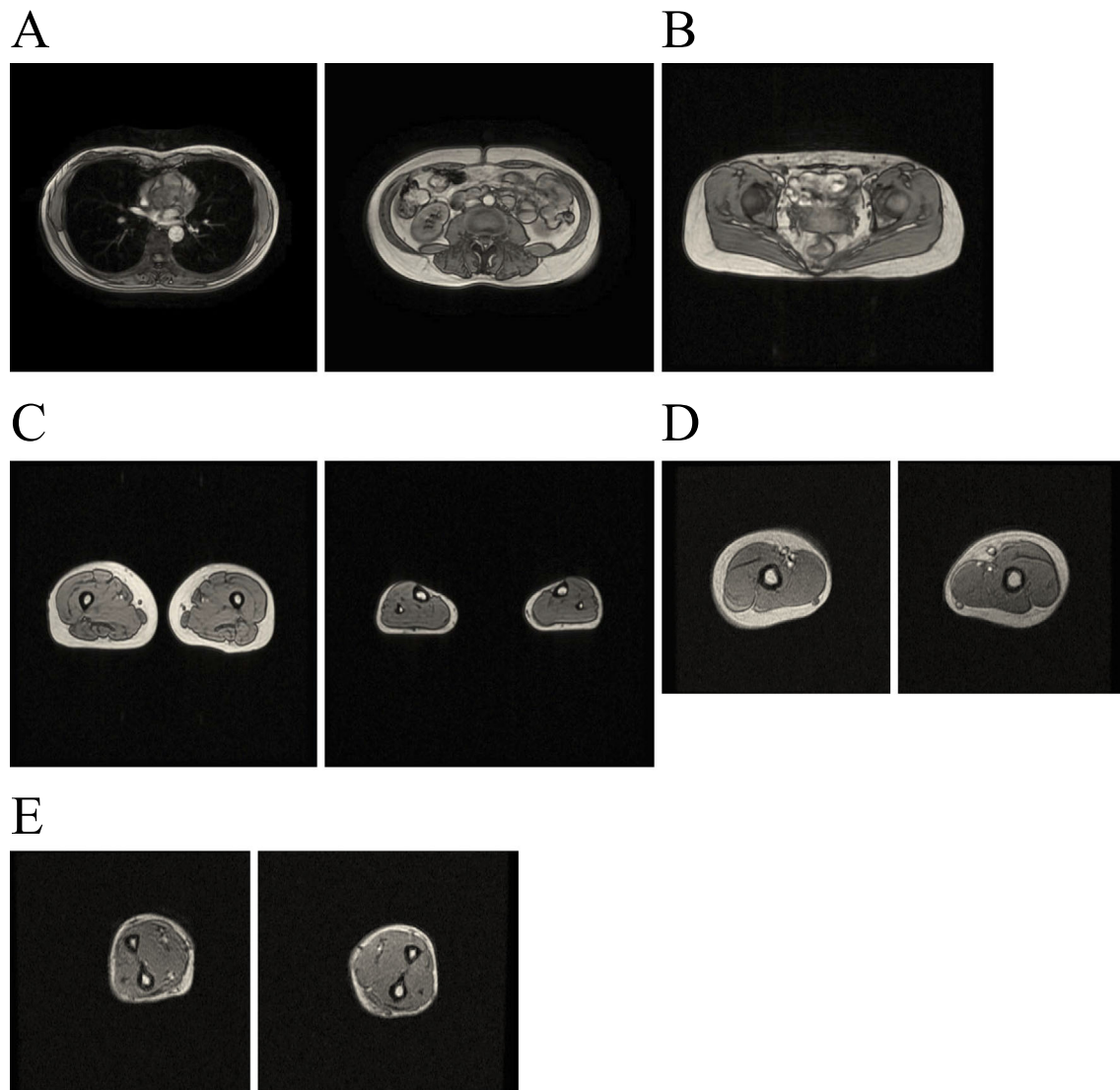


Figure 1. The (healthy) control subject was a 54-year-old woman. Her height was 156.0 cm, and she weighed 54.5 kg with a BMI of 22.4 kg/m². **A:** Thoracic MRI scans at the level of the seventh thoracic vertebrae (left panel) and abdominal MRI scans at the umbilical level (right panel) are shown. **B:** T1-weighted MRI scans at the middle level of the gluteus are shown. **C:** MRI scans at the middle level of the thigh (left panel) and calf (right panel) are shown. She had preserved subcutaneous fat in the entire circumference of the thigh and the calf. **D:** MRI scans at the middle level of the right arm (left panel) and left arm (right panel) are shown. **E:** MRI scans at the middle level of the right forearm (left panel) and left forearm (right panel) are shown. She had preserved subcutaneous fat in the entire circumference of the arm and the forearm.

shown in Fig. 5A and B, while nine patients who did not undergo a genetic analysis also had near-complete loss of subcutaneous fat, particularly in the antero-lateral and posterior thigh region and the calf region (data not shown).

As shown in Table 3, on DEXA, the fat mass of the lower limbs in Patient 4 was 3.9 kg, whereas this value was 1.7 kg in Patient 1, 2.3 kg in Patient 2, and 2.4 kg in Patient 3, as we reported previously (8-10). Thus, DEXA showed relatively low fat loss in the lower limbs of this patient compared with the others (Patients 1, 2, and 3) who had marked fat loss in the lower limbs. This is due to the fact that the amounts of subcutaneous fat in the internal thigh region were preserved in Patient 4 more abundantly than in Patients

1, 2, or 3, as shown in Figs. 5A and 2D. We must therefore bear in mind that patients may have PL even if they have relatively little fat loss in the lower limbs as estimated by DEXA.

Of note, Patient 5, who had relatively severe insulin resistance on the OGTT, as shown in Table 4, had near-complete loss of subcutaneous fat in the entire circumference of the calf despite having very low subcutaneous fat in the antero-lateral thigh region (Figs. 5A, 5B and 3C). We consider this to be an important finding for the diagnosis. In general, the amount of subcutaneous fat in the antero-lateral thigh region is lower than in the internal region. Therefore, it is sometimes difficult to distinguish between patients with and with-

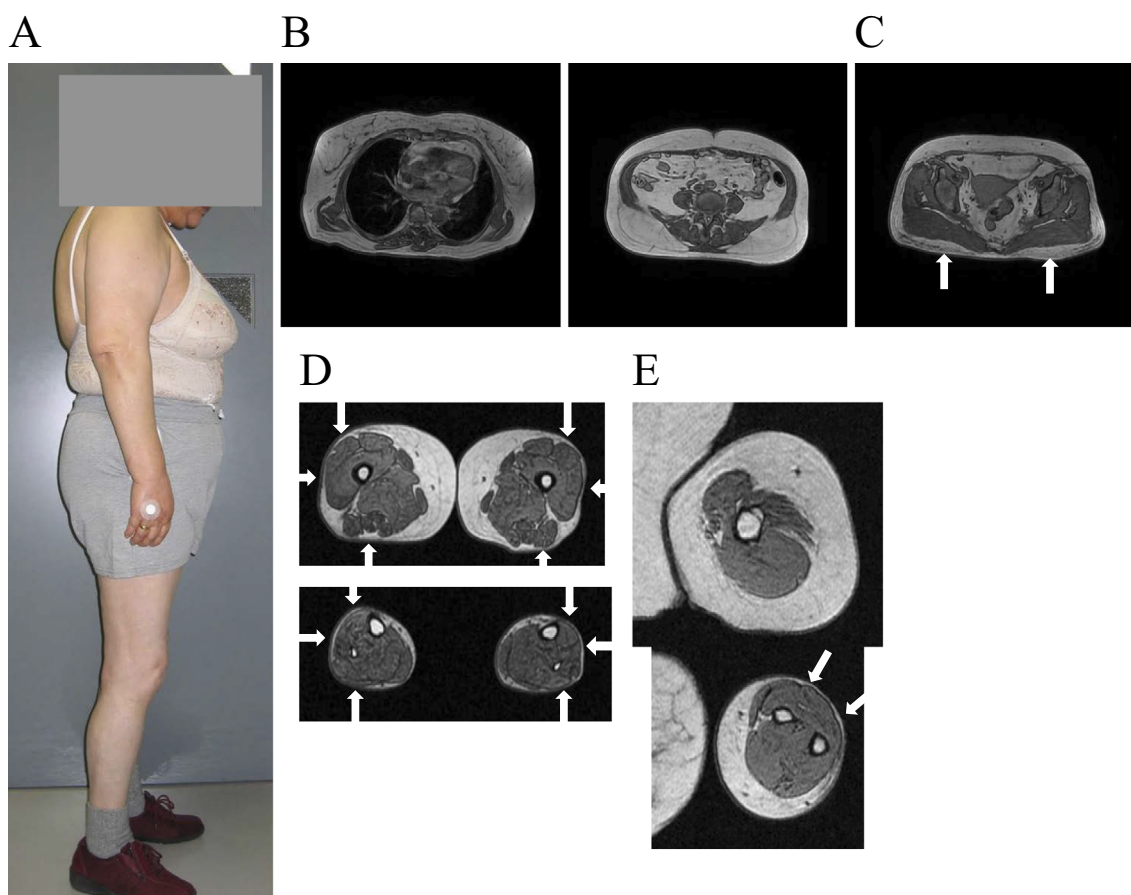


Figure 2. Patient 4 was a 69-year-old woman with a BMI of 25.5 kg/m². A: The phenotypical features of Patient 4 are shown in the left panel. She had loss of subcutaneous fat deposits in the forearm, lower limbs, and buttocks, with prominent lower limb musculature. She had excess fat deposition around the face, neck, and trunk, although that around the face and neck has been covered with gray paper for privacy. B: Thoracic MRI scans at the level of the seventh thoracic vertebrae (left panel) and abdominal MRI scans 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 the patient. C: T1-weighted MRI scans at the middle level of the gluteus are shown. MRI scans at the middle level of the gluteus in the proband revealed a decreased amount of gluteal subcutaneous fat, indicated by arrows, compared with the subcutaneous fat in the abdominal wall, indicating mild fat loss in the buttocks. She had a decreased amount of gluteal subcutaneous fat compared with control subjects with a mean BMI of 22.4 kg/m², as shown in Fig. 1. D: MRI scans at the middle level of the thigh (upper panel) and calf (lower panel) in the patient are shown. The patient had marked loss of subcutaneous fat, particularly in the antero-lateral and posterior thigh regions, which indicated by arrows, although subcutaneous fat in the internal thigh region remained. She had near-complete loss of subcutaneous fat in the antero-lateral and posterior calf regions, indicated by arrows, although very little subcutaneous fat in the internal calf region remained. E: MRI scans at the middle level of the left arm (upper panel) and forearm (lower 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 almost complete absence of subcutaneous fat in the antero-lateral forearm, indicated by arrows, although the preservation of subcutaneous fat in the internal forearm was observed.

out lipodystrophy based solely on the fat distribution in the antero-lateral thigh region, as some patients have low amounts of subcutaneous fat in the antero-lateral thigh region. Garg reported that one FPLD patient (FPLD3) with a PPARG mutation and another (FPLD1) with an unknown gene mutation had decreased amounts of subcutaneous fat, particularly in the anterior, lateral, and posterior regions of

the thigh and calf, sparing the subcutaneous fat in the medial parts (3). In our experience, the near-complete loss of subcutaneous fat in the calf region in patients suspected of having FPLD supports a diagnosis of lipodystrophy.

Subcutaneous fat loss in gluteal regions and forearms was accompanied by fat loss in the lower limbs in some patients. Recently, Pinnick reported that fat in the upper thighs and

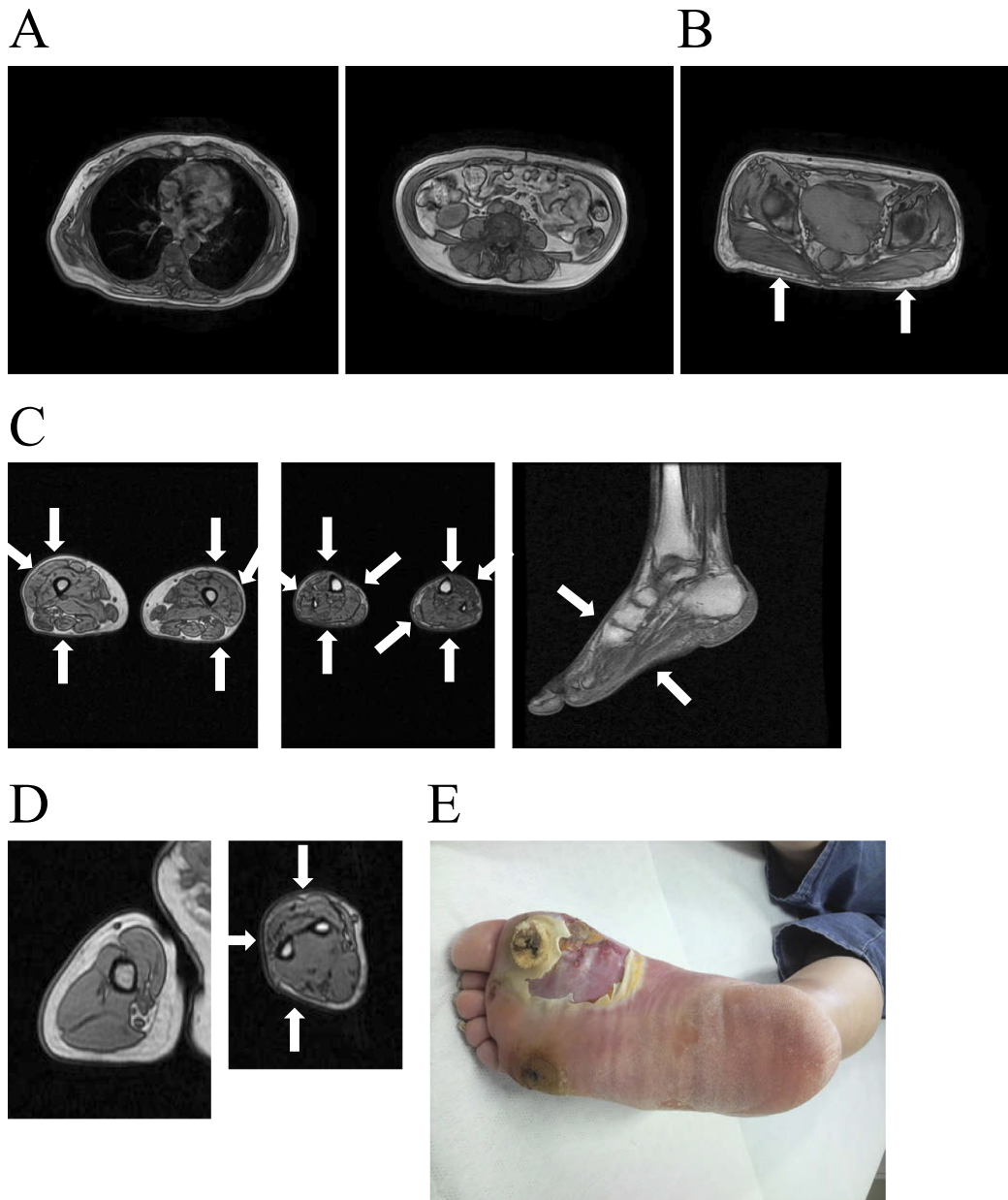


Figure 3. Patient 5 was a 50-year-old woman with a BMI of 22.2 kg/m². When she came to our hospital for the treatment of a diabetic ulcer (Fig. 4E), she was found to have diabetes. A: Thoracic MRI scans at the level of the seventh thoracic vertebrae (left panel) and abdominal MRI scans at the umbilical level (right panel) are shown. Thoracic and abdominal MRI revealed the preservation of subcutaneous fat in the thoracic and abdominal regions of Patient 5. B: T1-weighted MRI scans at the middle level of the gluteus are shown. MRI scans at the middle level of the gluteus in the proband revealed a decreased amount of gluteal subcutaneous fat, indicated by arrows, compared with control subjects with a BMI of 22.4 kg/m², as shown in Fig. 1. C: MRI scans at the middle level of the thigh (left panel) and calf (middle panel) in the patient are shown, as are scans at the level of the right leg (right panel). The patient had very little 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 near-complete loss of subcutaneous fat in the entire circumference of the calf, indicated by arrows. MRI scans of right leg revealed almost complete absence of dorsal and plantar subcutaneous fat, indicated by arrows. D: MRI scans at the middle level of the right arm (left panel) and forearm (right panel) are shown. Axial MRI at the middle level of the arm revealed the preservation of subcutaneous fat. Axial MRI at the level of the forearm revealed almost complete absence of subcutaneous fat in the antero-lateral and posterior forearm regions, indicated by arrows, although small amounts of subcutaneous fat in the internal forearm were preserved. E: The diabetic ulcer at her first visit is shown.

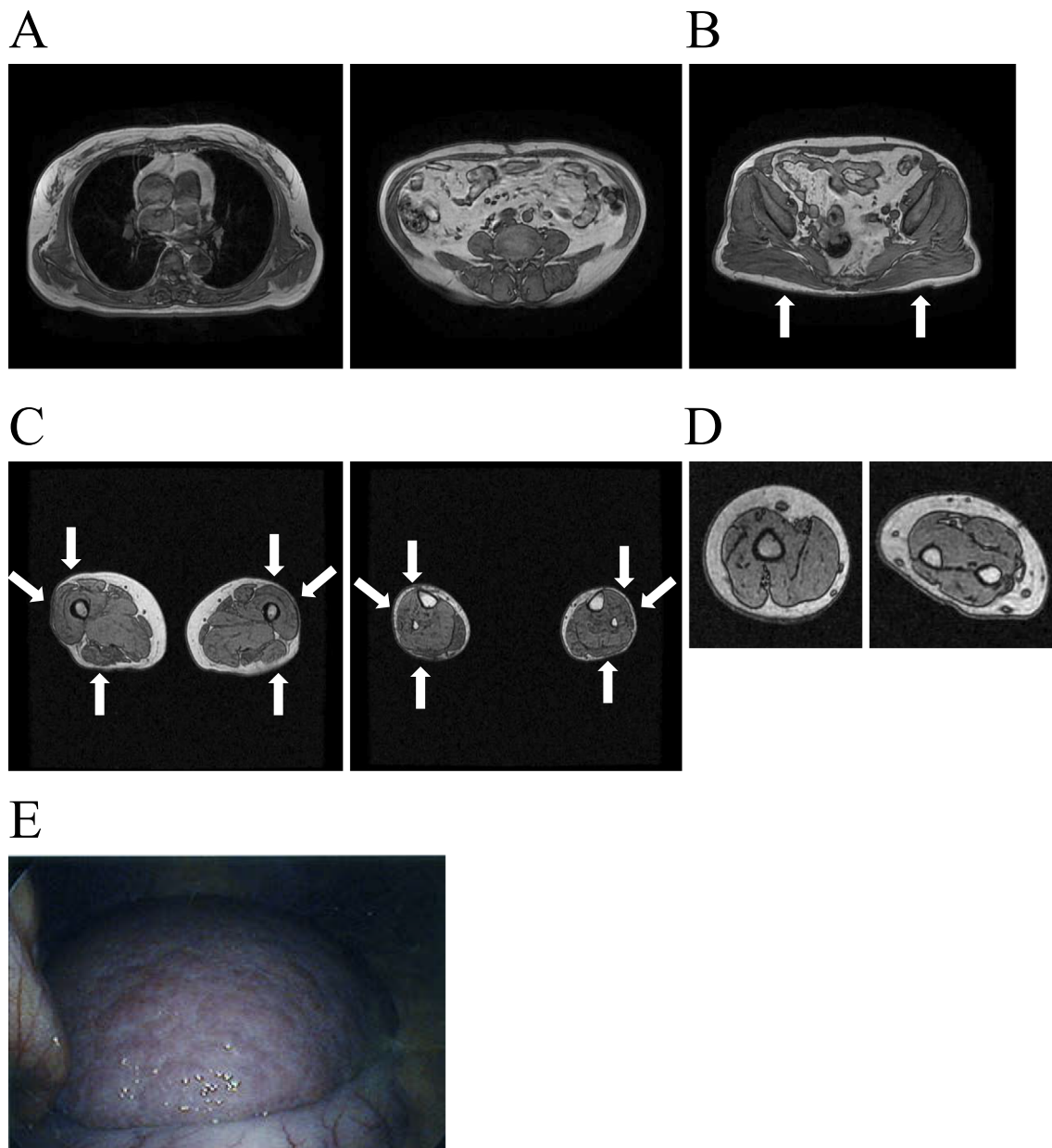


Figure 4. Patient 6 was a 67-year-old woman with a BMI of 25.2 kg/m². A: Thoracic MRI scans at the level of the sixth thoracic vertebrae (left panel) and abdominal MRI scans at the umbilical level (right panel) are shown. Thoracic and abdominal MRI revealed the preservation of subcutaneous fat in the thoracic and abdominal regions of Patient 6. B: T1-weighted MRI scans at the middle level of the gluteus are shown. MRI scans at the middle level of the gluteus in the proband revealed a decreased amount of gluteal subcutaneous fat, indicated by arrows, compared with control subjects with a mean BMI of 22.4 kg/m², as shown in Fig. 1. C: MRI scans at the middle level of the thigh (left panel) and calf (right panel) in the patient are shown. The patient had 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 marked loss of subcutaneous fat in the antero-lateral and posterior calf regions, indicated by arrows, although little subcutaneous fat in the internal calf region remained. D: MRI scans at the middle level of the right arm (left panel) and forearm (right panel) are shown. Axial MRI at the middle level of the arm revealed the preservation of subcutaneous fat, as did that at the middle level of the forearm. E: Nodular formation on the surface of the enlarged left hepatic lobe, consistent with liver cirrhosis or pre-cirrhosis, was shown by peritoneoscopy when Patient 6 underwent laparoscopic cholecystectomy for cholelithiasis at 67 years of age.

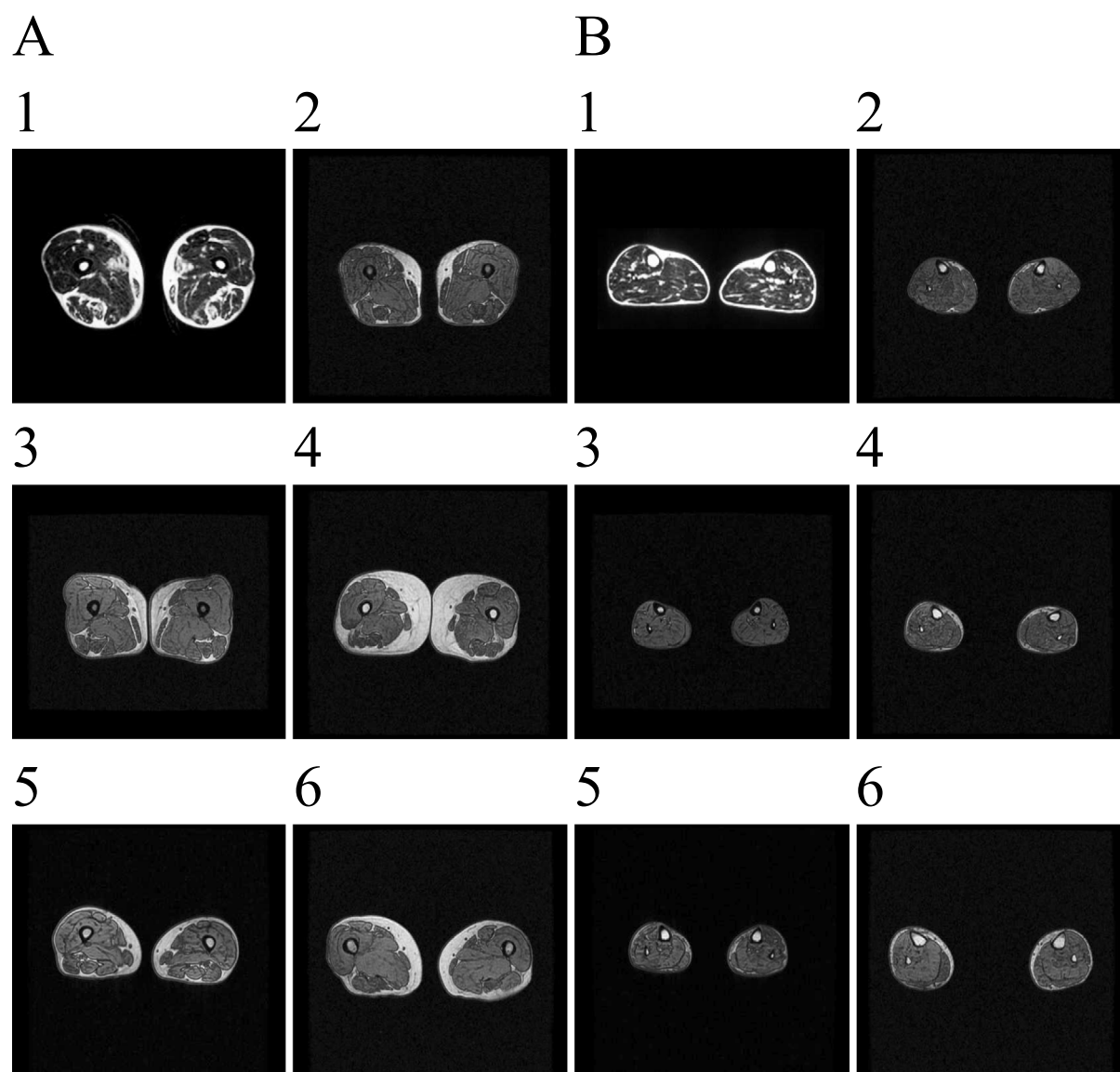


Figure 5. A: MRI scans at the middle level of the thigh in six patients are shown. All patients had near-complete loss of subcutaneous fat in the antero-lateral and posterior thigh region, although the amounts of subcutaneous fat in the internal thigh region were preserved in these patients. B: MRI scans at the middle level of the calf in six patients are shown. Patient 1 had very little subcutaneous fat in the entire circumference of the calf, while Patients 2, 3, and 5 had near-complete loss of subcutaneous fat in the entire circumference of the calf. Patients 4 and 6 had near-complete loss of subcutaneous fat in the calf region, particularly in the antero-lateral and posterior calf regions, although they had little subcutaneous fat in the internal calf region.

hips was negatively correlated with insulin resistance in 3,399 healthy subjects, and a microarray analysis showed that gluteal subcutaneous fat is protective against obesity-associated metabolic complications (14, 15). These data therefore suggest that decreased subcutaneous fat in the thigh and gluteal regions played an important role in insulin resistance and diabetes in our patients.

Genetic analyses showed that Patient 1 had no mutations in the genes LMNA, PPARG, AKT2, or Caveolin-1, while Patient 2 had no mutations in the genes LMNA, PPARG, or Caveolin-1. Genetic mutations in LMNA, PPARG, PLIN1, CIDEC, LIPE, ADRA2, and AKT2 have been reported to be

associated with FPLD (1-4). Whole-exome sequencing showed that Patients 3, 4, 5, and 6 had no mutations in these genes. Lotta et al. also reported that whole-genome of nine patients with FPLD1 revealed no mutations in candidate genes known to be associated with FPLD. Using 53 genomic regions, those authors showed a polygenic contribution to FPLD1 patients as well as to women with low leg fat mass in the general population, suggesting shared molecular mechanisms between these two groups (16). As women with FPLD1 had more severe fat loss in the lower limbs than expected from the relationship between the 53 single nucleotide polymorphism (SNP) genetic score and leg

Table 4. Oral Glucose Tolerance Test in Patients with Partial Lipodystrophy (PL).

Patient	BMI (kg/m ²)		Time (min)			
			0	30	60	120
1	22.9	Plasma glucose (mg/dL)	245	342	409	401
		Plasma insulin (μU/mL)	17.7	31.3	79.7	33.0
4	25.5	Plasma glucose (mg/dL)	98	167	218	293
		Plasma insulin (μU/mL)	7.9	24.6	29.9	44.9
5	22.2	Plasma glucose (mg/dL)	127	179	249	262
		Plasma insulin (μU/mL)	12.8	27.3	54.2	157.6
6	25.2	Plasma glucose (mg/dL)	95	175	177	140
		Plasma insulin (μU/mL)	8.8	55.5	70.1	39.7

fat mass in their study, the authors considered that additional genetic and environmental factors might contribute to FPLD1. Therefore, determining the 53 SNP genetic score in patients with PL may not necessarily help confirm a diagnosis of FPLD1. In addition, further studies are needed in order to determine the polygenic contribution to Japanese patients with FPLD1 and Japanese women with low leg mass in the general population. These 53 genomic regions include 11 insulin resistance risk alleles that are associated with lower gluteofemoral fat mass and three diseases of metabolic syndrome, namely hypertension, coronary artery disease, and type 2 diabetes (17, 18). Taken together, these findings suggest that our patients may have some mutations in novel genes related to FPLD or polygenic disorders with unknown additional genetic factors, resulting in FPLD1.

Guillin-Amarelle et al. recently reported that FPLD1, also known as Kobberling-type, is usually autosomal dominant, but its genetic cause is unknown and is characterized by fat loss in the lower limbs with abdominal fat accumulation, a high rate of positivity for a family history (90%), and metabolic disturbance (7). They performed a mutation analysis of the genes LMNA, PPARG, and LIPE but found no mutations in these genes. Although they did not sequence all of the FPLD-related genes, they considered that their patients might have FPLD1 with an unknown genetic cause, based on the clinical characteristics and lipodystrophic phenotype. In addition, they reported that decreased fat levels in the lower limbs in FPLD1 determine the severity of metabolic disturbances, including blood glucose levels and microvascular complications. All of our patients had subcutaneous fat loss in their lower limbs that was frequently accompanied by excess subcutaneous fat in the trunk. They also noticed fat loss during adolescence and had a family history of lipodystrophy. They had no mutations in candidate genes known to be associated with FPLD. Thus, our findings suggest that these patients had FPLD1, although diagnostic criteria for FPLD1 have not yet been established.

The levels of insulin deficiency and insulin resistance in Patient 4 were similar to those in type 2 diabetic patients, although Patients 1, 5, and 6 exhibited relatively severe insulin resistance. Patients 2 and 3 had severe insulin deficiency and were therefore treated with drugs such as sulfon-

ylurea, TZDs, GLP-1 receptor agonists, and insulin. It has been reported that TZDs, GLP-1 receptor agonists, and sodium-glucose cotransporter 2 (SGLT2) inhibitors are effective for treating lipodystrophic diabetes (19-21). However, we stopped the administration of TZDs in two patients because we observed marked weight gain during TZD treatment. Lipodystrophy is typically accompanied by low levels of leptin and adiponectin (22). Serum leptin was <10 ng/mL in 2 patients but increased to >10 ng/mL in 3 patients. As leptin replacement is effective for achieving glycemic control in lipodystrophic patients with low leptin levels as well as in those with normal or increased leptin levels (23-25), leptin replacement may be effective for treating diabetes in our patients.

Four of 6 patients had severe atherosclerosis, and 5 had a visceral fat area at the umbilical level of ≥ 100 cm² and were frequently accompanied by hypertriglyceridemia. This may be because our diabetic patients with PL were predisposed to fat accumulation in the remaining adipose tissue, including the visceral fat. The serum adiponectin levels was <5.1 μg/mL in 2 patients, and 4 patients had hypertension. Therefore, atherogenic risk factors and other factors, including unidentified causative genes, may be related to the development of atherosclerosis (9, 10, 26).

As shown in Fig. 4E, Patient 6 was highly suspected of having liver cirrhosis. She did not drink alcohol regularly, nor did she have any causes of liver cirrhosis, including viral infection and autoimmune hepatitis. As patients with PL are predisposed to fat accumulation in the remaining adipose tissues and other organs, including the liver, and given that she had marked accumulation of visceral fat, the liver cirrhosis in Patient 6 was likely caused by nonalcoholic steatohepatitis (NASH). Some patients with PL may be predisposed to develop NASH (23). Therefore, we need to monitor the liver function intensively and perform a liver biopsy at an early stage to diagnose NASH as soon as possible in patients with PL. Body weight reduction is advised in such patients, and TZDs, GLP-1 receptor agonists, SGLT2 inhibitors, and leptin may be beneficial regimens for managing NASH (20, 21, 27, 28). We must endeavor to prevent the development of NASH in diabetic patients with PL and NASH using these regimens.

There were three PL patients with a lower limb lean mass (Patients 4, 5, and 6) less than mean-2SD of Control 1. Some patients with diabetes have muscle atrophy caused by peripheral neuropathy, including autonomic dysfunction, insufficient blood supply, metabolic disturbance (e.g., poor glycemic control), unknown mechanisms, and diabetic amyotrophy (29). As our patients in the present study had noticed no change in the size of their lower extremities since adolescence and exhibited no neurological symptoms, including muscle weakness, the decreased lean mass of lower limbs in these five patients was likely not caused by these factors. As overlapping syndromes characterized by partial lipodystrophy and muscular dystrophy have recently been reported in association with unknown genetic origins (30), these patients may have had muscular dystrophy caused by unknown lipodystrophic genes. Therefore, the decreased lower limb lean mass in these patients may have been caused by unknown lipodystrophic genes.

The current study has several limitations. First, as we did not perform whole-exome sequencing in all patients, Patients 1 and 2 may have had mutations in candidate genes. Second, as we performed whole-exome sequencing in only four cases with FPLD, further studies involving whole-exome sequencing with more patients will be needed in order to clarify the prevalence and clinical characteristics of FPLD, including FPLD1.

In conclusion, all six of our patients with PL identified in our Japanese diabetic outpatient clinic may have FPLD1, suggesting that FPLD1 is the most common subtype of FPLD among diabetic Japanese women. The mean age at the diagnosis of diabetes in 6 patients with PL was 41.7 years, suggesting that diabetes may be diagnosed at a younger age in patients with PL than in those with type 2 diabetes. We herein report that patients with PL are not rare and can be found in a typical diabetic outpatient clinic. PL is reportedly often underdiagnosed in clinical practice (31). Importantly, to detect PL, clinical practitioners should pay careful attention to subcutaneous fat loss in individual regions of the body, particularly the lower limbs. When we have difficulty diagnosing patients with incomplete fat loss with FPLD1, determining the 53 SNP genetic scores may not necessarily help confirm the diagnosis as FPLD1. Therefore, further studies are required to clarify the precise molecular mechanism of pathogenesis and confirm the diagnostic criteria for FPLD1. Patients with PL often have severe atherosclerosis (9, 10, 26). The decreased fat levels in lower limbs in FPLD1 may determine the severity of metabolic disturbances, including the blood glucose level, microvascular complications, and cardiovascular complications (7). We should try to detect patients with PL, including FPLD1, earlier in order to achieve glycemic control and prevent atherosclerosis and NASH in this population.

The authors state that they have no Conflict of Interest (COI).

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