Contents available at ScienceDirect

Diabetes Research and Clinical Practice

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

International Diabetes Federation

Clinical characteristics in two patients with partial lipodystrophy and Type A insulin resistance syndrome due to a novel heterozygous missense mutation in the insulin receptor gene

Masanori Iwanishi [a,*], *Toru Kusakabe* [b], *Choka Azuma* [a], *Yuji Tezuka* [a], *Yukako Yamamoto* [a], *Jun Ito-Kobayashi* [a], *Miki Washiyama* [a], *Mayumi Morimoto* [c], *Ken Ebihara* [d]

[a] *Department of Diabetes and Endocrinology, Kusatsu General Hospital 1660 Yabase, Kusatsu, Shiga 525-8585, Japan*
[b] *Department of Endocrinology, Metabolism and Hypertension, Clinical Research Institute, National Hospital Organization Kyoto Medical Center 1-1 Fukakusa Mukaihata-cho, Fushimi-ku, Kyoto 612-8555, Japan*
[c] *Department of Pediatrics, Kusatsu General Hospital, 1660 Yabase, Kusatsu, Shiga 525-8585, Japan*
[d] *Division of Endocrinology and Metabolism, Jichi Medical University 3311-1 Yakushiji, Shimotsuke, Tochigi 329-0498, Japan*

ARTICLE INFO

Article history:
Received 28 February 2019
Received in revised form
9 April 2019
Accepted 30 April 2019
Available online 16 May 2019

Keywords:
Type A insulin resistance syndrome
Insulin receptor gene mutation
Partial lipodystrophy
Polycystic ovary syndrome

ABSTRACT

Aims: The present report aimed to clarify the clinical characteristics in a girl at the age of 12 and her mother with partial lipodystrophy and Type A insulin resistance syndrome.
Methods: We examined fat distribution in the patients using dual-energy X-ray absorptiometry, magnetic resonance imaging, and computed tomography. We performed genetic analysis to examine the causal gene for lipodystrophy and insulin resistance.
Results: Both patients had partial lipodystrophy and a novel heterozygous missense mutation ($Asn^{1137} \rightarrow Lys^{1137}$) in the insulin receptor gene. Because Asn^{1137} in the catalytic loop is conserved in all protein kinases, this mutation was thought to impair insulin receptor function. By whole-exome sequencing, we found the proband had neither mutations in candidate genes known to be associated with familial partial lipodystrophy nor novel likely candidate causal genes. Taken together, we thought that fat loss in these two patients might be caused by insulin receptor dysfunction. The proband had amenorrhea due to polycystic ovary syndrome. Her menstruation improved, as fat loss was restored during adolescence. This might be caused by improving insulin resistance due to increased levels of leptin and fat mass.
Conclusions: This case might help to understand the mechanisms insulin receptor dysfunction that cause lipodystrophy.

* Corresponding author.
E-mail address: masa-iwani@solid.ocn.ne.jp (M. Iwanishi).
https://doi.org/10.1016/j.diabres.2019.04.034
0168-8227/

1. Introduction

Severe insulin resistance syndromes (SIRS) are a complex group of disorders with impaired cellular responsiveness to insulin signaling. SIRS are grouped into three categories, involving lipodystrophy, primary insulin signaling defect (e.g. insulin receptor mutation), and complex syndromes associated with insulin resistance (e.g. POLD1 mutation) [1]. Type A insulin resistance syndrome (IRS) is a rare form of SIRS that is frequently caused by insulin receptor dysfunction, which is due to mutations in the insulin receptor gene [2,3]. Type A IRS is characterized by insulin-resistant glucose intolerance, acanthosis nigricans, and hyperandrogenism. Female patients with Type A IRS often have hirsutism and oligomenorrhea with polycystic ovaries, which result from hyperandrogenism due to severe insulin resistance. Various mutations in the insulin receptor have been identified in Type A IRS. Patients with Donohue syndrome, or Rabson–Mendenhall syndrome, with homozygous or compound heterozygous mutations in the α-subunit have more severe insulin resistance and are sometimes lipodystrophic [4,5]. In contrast, it has been reported that patients with heterozygous loss-of-function in the insulin receptor are not commonly lipodystrophic [4,6]. It has been also reported that adipocyte-specific insulin receptor knockout mice showed lipodystrophy in an animal model [7]. Taken together, as it was conceivable that insulin receptor function plays an important role in adipocyte differentiation, the lipodystrophy in Type A IRS may be caused by insulin receptor dysfunction. However, to the best of our knowledge, there are no reports examining the precise fat distribution in patients with Type A IRS due to a heterozygous missense mutation in the insulin receptor gene.

In this paper, we present a case of a 12-year-old diabetic girl and her mother with partial lipodystrophy and Type A IRS due to a novel heterozygous missense mutation ($Asn^{1137} \rightarrow Lys^{1137}$) in the insulin receptor gene.

2. Subjects and methods

2.1. Study subjects

The proband (Subject 1) was a 12-year-old girl with severe insulin resistance, and her mother (Subject 2) was a 44-year-old woman with relatively severe insulin resistance. Her father (Subject 3) was a 41-year-old man with type 2 diabetes. A control group (Control 1) with 41 healthy female volunteers between 40 and 47 years of age was recruited from the employees of Tanita, Inc. Another control group (Control 2) with 36 healthy male volunteers between 40 and 49 years of age was also recruited from among the employees of Tanita. Inc. A third control group (Control 3) with 64 girls aged 11 years old was recruited from among healthy elementary school children. The mean body mass index (BMI) in Control groups 1, 2, and 3 was 22.5, 24.5, and 17.8 kg/m^2, respectively. We evaluated the body composition as determined by dual-energy X-ray absorptiometry (DEXA) in Subject 1, Subject 2, and Subject 3 using Control 3, Control 1, and Control 2, respectively.

This study (approved on 2 September 2016, approval number: 2016090203), including whole-exome sequencing (approved on 12 December 2016, approval number: 2016120206) was approved by the ethics committee of Kusatsu General Hospital. The subjects agreed to participate in the study and provided their verbal informed consent for the publication of this report.

2.2. Methods

2.2.1. 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, hemoglobin A1c (HbA1c), serum insulin level, serum leptin level, and serum adiponectin level were measured as reported previously [8,9].

2.2.2. 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 min.

2.2.3. Hyperinsulinemic-euglycemic glucose clamp technique

Insulin action on glucose uptake in peripheral tissues was evaluated using the hyperinsulinemic-euglycemic glucose clamp technique [10].

2.2.4. Dual-energy X-ray absorptiometry

Whole-body DEXA was performed with a multiple detector fan-beam Hologic QDR-4500W densitometer (Hologic, Marlborough, USA) in the subject group and with a multiple detector fan-beam GE Lunar DPXL densitometer (General Electric, Fairfield, USA) in the control groups.

2.2.5. Magnetic resonance imaging

Magnetic resonance imaging (MRI) was performed using a 3.0 Tesla imaging device (Sigma Horizon; General Electric). 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.

2.2.6. Evaluation and measurement of fat distribution by computed tomography scanning

Fat was easily identified on computed tomography (CT) scans because of its relatively low signal intensity on images compared with other tissues such as muscle. Subcutaneous and visceral fat areas were measured using a −150 to 50– Hounsfield unit area with a modified CT method (Light Speed Plus-R; General Electric), as described by Tokunaga et al., at the umbilical level [11].

2.2.7. Genetic analysis

We obtained written informed consent for genetic analyses from the patients for the genetic analyses. The 22 exons of the insulin receptor gene and its intron–exon junctions were amplified by PCR using 21 pairs of primers [12]. Then, the

PCR products were sequenced. We performed whole-exome sequencing for the proband as reported previously [8,9].

2.2.8. Three-dimensional model structure of insulin receptor

The three-dimensional (3D) model structure of insulin receptor tyrosine kinase was prepared by PyMOL [13].

3. Results

3.1. Case report

Family tree, biochemical data, and OGTT in the family are shown in Fig. 1A, Table 1, and Table S1. We presented the results of OGTT in healthy young Japanese women and men in Table S1 [14]. Body composition as determined by DEXA scan in the family is shown in Table 2. The patient was a 12-year-old girl. She was seen by a pediatrician at Kusatsu General Hospital because her urine glucose was positive in an annual health check. Her HbA1c, fasting glucose level, and fasting insulin level were 7.1%, 76 mg/dL, and 83.8 μU/mL, respectively. She was admitted to our hospital because of the evaluation of diabetes with severe insulin resistance. Her height was 153.0 cm, and she weighed 39.0 kg with a BMI of 16.7 kg/m^2. She had acanthosis nigricans at the axilla and hirsutism on her back. A 75 g OGTT showed that her blood glucose pattern had impaired glucose tolerance, whereas her serum insulin levels at fasting and at 2 h after OGTT were 83.8 μU/mL and 1149 μU/mL, respectively, suggesting severe insulin resistance. Neither anti-insulin nor anti-insulin receptor antibodies were detected. Her serum leptin level was 3.9 ng/mL, whereas her serum adiponectin level was 17.3 μg/mL. As shown in Table 2, the percentages (%fat) in the total body fat, upper limbs, trunk, and lower limbs were 12.8%, 15.9%, 6.8%, and 19.0%, respectively. The total body fat, upper limbs, trunk, and lower limbs fat masses were 4.6, 0.6, 1.2, and 2.3 kg, respectively. The DEXA study showed that the ratio of truncal fat to whole body fat was 26.0%. As shown in Table S2, Kubo et al. reported the evaluation of partial body composition in 64 healthy 11-year-old girls, who had a mean BMI of 17.8 kg/m^2, using a bioelectrical impedance analysis method [15]. The approximate mean value of total percentages of body fat (%fat), upper limbs, trunk, and lower limbs were 20.5%, 18.0%, 17.0%, and 26.0%, respectively. An approximately mean values of total body fat, upper limbs, trunk, and lower limbs fat mass were 8.5, 0.6, 4.0, and 4.0 kg, respectively. The approximately mean ratio of truncal fat to whole body fat in the 64 girls was 47%. Therefore, compared with body composition in the 64 healthy control subjects, these body composition data in the proband suggested she had the decreased fat mass in her trunk and lower limbs, whereas the fat mass in the upper limbs seemed to be well preserved. As shown Fig. 2A 2 and 3 and Fig. 2B 1, subcutaneous fat in the thorax of the proband, indicated by yellow arrows, was as sparse as that in a control aged 13 years old and a BMI of 19.0 kg/m^2, indicated by blue arrows. However, this could not rule out that the proband did not have subcutaneous fat loss due to impairment of adipocyte differentiation in the thorax. As shown in Fig. 2A 4, 5, and 6 and Fig. 2B 2, 3, and 4, the proband had decreased subcutaneous fat in the abdomen, indicated by yellow arrows, compared with a control subject, indicate by blue arrows. In contrast, as shown Fig. 2A 7, 8, 9, and 10, the subcutaneous fat in the upper limbs and lower limbs seemed to be well preserved. However, as shown in Tables 2 and S2, the DEXA study

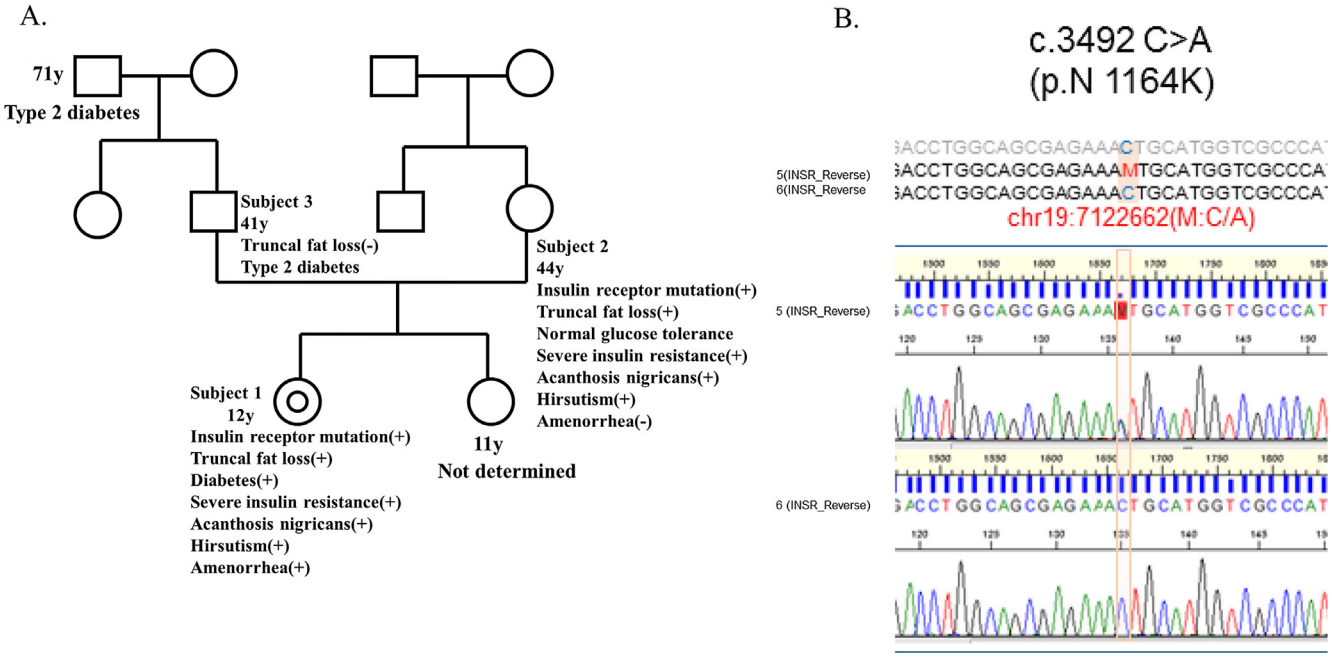

Fig. 1 – A: Clinical features in this family and family pedigree of the proband are shown. Subject 1: proband, Subject 2: her mother, Subject 3: her father B: The 22 exons of the insulin receptor gene and its intron–exon junctions were amplified by PCR using 21 pairs of primers [12]. Then, the PCR products were sequenced. The patient was heterozygous for a novel mutation, c.3492C > A (p.N1164K). This location (1164) corresponded to the number of amino acid 1137 in the report by Seino et al. [12].

Table 1 – Biochemical data of this family.

Subject	Age (years)	BMI (kg/m^2)	HbA1c (%)	Plasma glucose (mg/dL)	Plasma insulin (μU/mL)	Plasma CPR (ng/dL)	Serum cholesterol (mg/dL)	Serum triglyceride (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	12	16.7	7.1	76	83.8	2.4	152	73	59	17	9	39.5	3.9	17.3
2	44	18.3	5.4	73	20.3	0.8	167	50	73	14	11	15.0	4.7	15.5
3	41	29.0	6.3	114	12.0	ND	188	67	35	39	71	23.8	10.6	4.2
Normal values			4.6–6.2	70–109	2.7–10.4	1.1–4.4	130–219	30–149	40–90	8–38	4–43	13.0–36.9		

Subject 1: proband, Subject 2: mother, Subject 3: father.
SD: standard deviation AST: aspartate transaminase ND: not determined.
ALT: alanine transaminase PLTs: platelets.

showed that the proband had decreased fat mass in her lower limbs, whereas she had similar fat mass in the upper limbs compared with the control subjects. Therefore, we considered that she had decreased subcutaneous fat in her lower limbs. Abdominal CT scans of the proband showed that the subcutaneous fat area and visceral fat area in the umbilical portion were 37.7 cm^2 and 35.1 cm^2, respectively, suggesting decreased subcutaneous fat and visceral fat in her abdomen (data not shown). Taken together, we considered that she had subcutaneous fat loss in her abdomen and lower limbs, possible subcutaneous fat loss in her thorax, and preserved fat mass in her upper limbs, suggesting that she had partial lipodystrophy. After we found that the patient had partial lipodystrophy with insulin resistance at Kusatsu General Hospital, she was transferred to Kyoto University Hospital for further examinations. She was admitted to Kyoto University Hospital for monitoring at the ages of 12, 14, and 16 years. Peripheral insulin sensitivity indices were evaluated via euglycemic-hyperinsulinemic clamp, and her glucose infusion rate (GIR) at the ages of 12, 14, and 16 years was 4.93, 4.89, and 3.45 mg/kg/min, respectively, which suggested that she had severe insulin resistance. At the age of 14 years, her serum follicular stimulating hormone (FSH), serum luteinizing hormone (LH), and testosterone levels were 2.5, 10.9, and 117.2 ng/dL, respectively. The normal ranges in the levels of serum FSH, serum LH, and serum testosterone are 2.0–19.3, 3.9–57.3, and 10–86 ng/dL, respectively; therefore, her serum testosterone level was high and her serum LH and FSH levels were within the normal ranges, although the ratio of LH to FSH was >1.0. An MRI study of the pelvis revealed that her ovaries were enlarged and polycystic. Thus, she was diagnosed with polycystic ovary syndrome.

The proband's mother was a 44-year-old woman. Her height was 162.0 cm, and she weighed 48.0 kg with a BMI of 18.3 kg/m^2. She also had acanthosis nigricans at the axilla and hirsutism on her back. Her HbA1c was 5.4%. OGTT showed that her blood glucose pattern was normal glucose tolerance, whereas her serum insulin levels at fasting and at 2 h after OGTT were 20.3 μU/mL and 151.2 μU/mL, respectively, suggesting relatively severe insulin resistance. Neither anti-insulin nor anti-insulin receptor antibodies were detected. Her serum leptin level was 4.7 ng/mL, whereas her serum adiponectin level was 15.5 μg/mL. As shown in Table 2, the percentages (%fat) in her total body fat, upper limbs, trunk, and lower limbs were 24.5%, 22.5%, 15.6%, and 37.1%, respectively. Her total body fat, upper limbs, trunk, lower limbs fat masses were 11.5, 1.1, 3.2, and 6.6 kg, respectively. The DEXA study showed that her ratio of truncal fat to whole body fat was 27.8%. In contrast, the percentage (%fat) of total body fat, upper limbs, trunk, lower limbs in 41 control women, mean age 43.9 years and BMI 22.5 kg/m^2, was 30.0%, 26.6%, 27.8%, and 33.1%, respectively. The total body fat, upper limbs, trunk, lower limbs fat masses of the controls were 16.6, 1.4, 6.9, and 7.0 kg, respectively. The DEXA study showed that their ratio of truncal fat to whole body fat was 40.4%. Therefore, compared with the 41 control women, the mother had decreased fat mass in the trunk, whereas the fat mass in the upper limbs and lower limbs seemed to be well preserved. As shown respectively in Figs. S1A–C and S1D–F, thoracic and abdominal CT scans showed that com-

Table 2 – Body composition as determined using DEXA scans in this family.

Sex	Subject 1 Female	Subject 2 Female	Subject 3 Male	Control 1 (n = 41) Female	Control 2 (n = 36) Male
Height (cm)	153.0	162.0	182.0	156.8 ± 4.7	168.2 ± 5.3
Body weight (kg)	39.0	48.0	96.0	55.1 ± 6.3	69.3 ± 9.0
BMI (kg/m^2)	16.7	18.3	29.0	22.5 ± 3.0	24.5 ± 2.9
Age (y)	12	44	41	43.9 ± 2.1	44.9 ± 2.7
(1) Fat (%)					
Total %fat	12.8	24.5	27.2	30.0 ± 5.8	20.8 ± 5.3
Upper limbs %fat	15.9	22.5	26.4	26.6 ± 6.8	16.0 ± 4.7
Trunk %fat	6.8	15.6	33.1	27.8 ± 7.6	22.9 ± 6.4
Lower limbs %fat	19.0	37.1	19.7	33.1 ± 5.2	19.7 ± 4.7
(2) Fat mass (kg)					
Total fat mass	4.6	11.5	25.0	16.6 ± 4.7	14.6 ± 4.9
Upper Limbs fat mass	0.6	1.1	2.4	1.4 ± 0.5	1.0 ± 0.2
Trunk fat mass	1.2	3.2	15.6	6.9 ± 2.8	7.3 ± 2.8
Lower limbs fat mass	2.3	6.6	5.6	7.0 ± 1.6	5.3 ± 0.9
(3) Lean mass (kg)					
Total lean mass	30.0	33.3	64.3	35.5 ± 3.1	51.3 ± 5.4
Upper limbs lean mass	3.0	3.4	6.3	3.4 ± 0.4	5.1 ± 0.3
Trunk lean mass	15.4	16.8	31.0	16.4 ± 1.4	22.9 ± 2.6
Lower limbs lean mass	9.1	10.5	21.7	13.2 ± 1.8	19.8 ± 1.4
(4) Trunk fat mass/ Total fat mass (%)	26.0	27.8	62.4	40.4 ± 7.1	49.2 ± 4.6

Subject 1: proband, Subject 2: mother, Subject 3: father.
Normal values in Control 1 were obtained from 41 healthy women between the ages of 40 and 47 years.
Normal values in Control 2 were obtained from 36 healthy men between the ages of 40 and 49 years.
Values in Control 1 and Control 2 were expressed as mean ± SD.

pared with subcutaneous fat in the abdomen in a control woman aged 40 years old and with a BMI of 19.1 kg/m^2, the mother had a decreased subcutaneous fat in the thorax and abdomen. Abdominal CT scans also showed that the subcutaneous and visceral fat areas in the umbilical portion were 69.9 cm^2 and 8.0 cm^2, respectively (data not shown), suggesting decreased subcutaneous fat and visceral fat in the abdomen. Taken together, these data suggested that the mother had truncal fat loss. She had a regular menstruation.

The proband's father was a 41-year-old man. His height was 182.0 cm, and he weighed 96.0 kg with a BMI of 29.0 kg/m^2. His HbA1c level was 6.3%. OGTT showed that his blood glucose pattern was diabetic without hyperinsulinemia during OGTT. His serum leptin level was 10.6 ng/mL, whereas his serum adiponectin level was 4.2 μg/mL. As shown Table 2, compared with control men with a mean age of 44.9 years and BMI of 24.5 kg/m^2, the DEXA study showed that he did not have decreased truncal fat mass.

3.2. *Genetic analysis in two patients*

Using sequence analysis of amplified genomic DNA, we demonstrated that the proband was heterozygous for a novel mutation, c.3492C > A (p.N1164K) in the insulin receptor gene, as shown in Fig. 1B. This location (1164) corresponds to amino acid 1137 in the report by Seino et al. [12]. In this report, we used the amino acid numbering established by Seino et al. Her mother also had the same novel mutation, c.3492C > A, (p.N1137K) in the insulin receptor gene (data not shown). Because the proband had partial lipodystrophy and genetic inheritance was thought to be autosomal dominant, we performed whole-exome sequencing to examine whether or not she had mutations in candidate genes known to be associated with familial partial lipodystrophy (FPLD), including LMNA, PPARG, PLIN1, CIDEC, LIPE, ADRA2, and AKT2 [8,9]. We found that she had neither mutations in candidate genes known to be associated with FPLD nor novel likely candidate causal genes except for the insulin receptor mutation.

3.3. *Changes in fat distribution, glucose intolerance, and menstrual function in two patients*

The HbA1c levels of the proband had been within the normal range since she received dietary therapy at the age of 12, except for approximately 3 months, in which it increased to 8.1%, when her body weight increased to 55.0 kg from 50.0 kg at the age of 20. Therefore, she always been mindful to maintain her body weight at 50 kg. At the age of 22, she was transferred to Kusatsu General Hospital again and has been visiting an outpatient clinic regularly. The changes in fat and lean mass determined using DEXA, serum leptin, and serum adiponectin levels are shown in Table 3. The truncal fat in the proband increased at the ages of 16 and 23 years, compared with her truncal fat at the ages of 12 and 14 years. Her lower limb fat also had increased during adolescence. Thus, her truncal fat and lower limbs fat increased as she grew up. As the truncal fat in the patient increased, the ratio of truncal fat to whole body fat and the levels of serum leptin increased at an age of 23 years. The levels of serum testosterone (normal range: 0.11–0.47 ng/mL) at the ages of 22, 23,

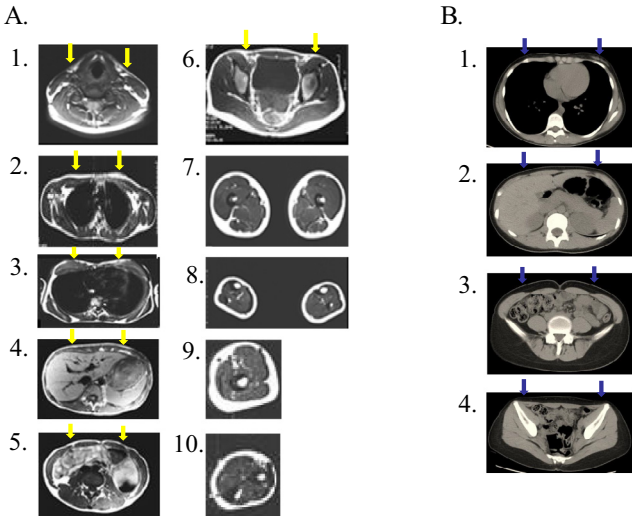

Fig. 2 – A: MRI scans in body portions of the patient are shown in 1–10. MRI scans at the level of the cervical region (1), chest (2, 3), upper abdomen (4), abdominal umbilical region (5), gluteus (6), thigh (7), calf (8), arm (9), and forearm (10) revealed fat distribution in the body and extremities. The MRI scans showed decreased subcutaneous fat in the anterior cervical region, chest, abdomen, and gluteus, indicated by yellow arrows, whereas subcutaneous fat in the upper limbs and lower limbs seemed to be well preserved. B: CT scans at the level of the lower thorax (1), upper abdomen (2), abdominal umbilical region (3), and gluteus (4) revealed fat distribution in the body in a control subject. The (healthy) control subject was a 13-year-old girl (height 155.3 cm; weight 45.6 kg; BMI 19.0 kg/m^2). As shown in Figure A 2, 3, and Figure B 1, subcutaneous fat in thorax in the proband, indicated by yellow arrows, was as sparse as that in a control girl aged 13 years old and a BMI of 19.0 kg/m^2, indicated by blue arrows. As shown in Figure A 4, 5, 6, and Figure B 2, 3, 4, the proband had decreased subcutaneous fat in abdomen, indicated by yellow arrows, compared with the subcutaneous fat in abdomen in a control subject, indicated by blue arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

24, and 25 years were 0.53, 0.53, 0.45, and 0.37, respectively. Thus, the levels of serum testosterone at an age of 24 years decreased to the normal range. Her menstruation gradually became more regular without any specific treatment after the age of 20.

4. Discussion

We present two rare case of a patient and her mother with partial lipodystrophy and heterozygous mutation in the insulin receptor in this report. Lipodystrophy is a condition characterized by regional or total selective loss or absence of subcutaneous fat in the absence of nutritional deprivation or catabolic state. The core finding for the diagnosis of lipodystrophy is the loss or absence of subcutaneous fat in a partial or generalized fashion. We showed the proband at the age of 12 had fat loss in the trunk and lower limbs,

Table 3 – Changes in fat and lean mass in the patient determined using DEXA, serum leptin, and serum adiponectin levels.

Age (y)	12	14	16	23
Height (cm)	153.0	155.0	158.2	158.0
Body weight (kg)	39.0	42.0	49.1	52.3
BMI (kg/m^2)	16.7	17.5	19.7	21.0
(1) Fat (%)				
Total %fat	12.8	19.1	20.0	34.3
Upper limbs %fat	15.9	28.0	22.4	41.1
Trunk %fat	6.8	7.4	12.5	29.3
Lower limbs %fat	19.0	33.0	27.7	42.9
(2) Fat mass (kg)				
Total fat mass	4.6	7.9	9.9	18.0
Upper limbs fat mass	0.6	1.2	1.1	2.1
Trunk fat mass	1.2	1.4	2.5	7.4
Lower limbs fat mass	2.3	4.6	5.1	7.6
(3) Lean mass (kg)				
Total lean mass	30.0	31.7	37.7	32.2
Upper limbs lean mass	3.0	2.9	3.5	2.7
Trunk lean mass	15.4	17.0	17.3	17.2
Lower limbs lean mass	9.1	8.9	12.8	9.5
(4) Trunk fat mass/ Total fat mass (%)	26	18	26	43.3
(5) Leptin (ng/mL)	3.9			15.4
(6) Adiponectin (μg/mL)	17.3			14.4

whereas her mother had fat loss in the trunk using DEXA and MRI evaluations, compared with control subjects. Thus, we diagnosed lipodystrophy in accordance with guidelines on the definitive criteria for the diagnosis of lipodystrophy [16,17]. Fat loss in the trunk and lower limbs in the proband recovered during adolescence, whereas her mother continued to have fat loss in the trunk from the age of 44 to her current age of 56. The proband's father did not have lipodystrophy, severe insulin resistance, or suppressed serum adiponectin level, and we considered that glucose intolerance in the father was due to type 2 diabetes. The proband and her mother had loss of subcutaneous fat in the trunk and loss of visceral fat in the abdomen. This fat distribution is atypical for FPLD, because it was reported that decreased subcutaneous fat in the lower limbs, lower limbs, and buttocks, or in the lower limbs, buttocks, and forearms, is characteristic of patients with FPLD [8,9,16]. Because the patient had partial lipodystrophy and the genetic inheritance was possibly thought to be autosomal dominant, she had a possibility that she had FPLD. Therefore, we performed whole-exome sequencing in the patient to examine whether or not she had mutations in candidate genes known to be associated with FPLD, including LMNA, PPARG, PLIN1, CIDEC, LIPE, ADRA2, and AKT2. We found that she had neither mutations in these genes nor novel likely candidate causal genes except for the insulin receptor mutation. This result suggested partial lipodystrophy in these two patients might be caused by insulin receptor dysfunction, because it is conceivable that the insulin receptor plays an important role in adipocyte differentiation [4,5,7].

Insulin receptor dysfunction in the liver results in a decrease in insulin-stimulated hepatic lipogenesis [18]. Therefore, patients with insulin receptor mutations exhibit

an absence of fatty liver, normal serum triglyceride, and normal high-density lipoprotein (HDL) cholesterol. In contrast, patients with FPLD, including AKT2, often exhibit femorogluteal lipodystrophy with fatty liver, hypertriglyceridemia through enhanced insulin-stimulated hepatic lipogenesis. and suppressed serum adiponectin. The levels of serum leptin were low in the patient and her mother, whereas the levels of serum adiponectin were high. The levels of serum leptin and adiponectin are usually low in patients with FPLD [19], although the levels of serum leptin are not low in some patients with FPLD1 [8,9]. It has been reported that the levels of serum adiponectin are high in patient with insulin receptoropathies, including insulin receptor mutation and type B insulin resistance [20].

In 2013, mutations in PIK3R1 were demonstrated to cause SHORT syndrome, denoting short stature, joint hyperextensibility, ocular depression, Rieger anomaly (a developmental defect in the iris), and teething delay [21–23]. There are common states between patients with insulin receptor mutation and those with PI-3 kinase subunit P85 mutation. They could have both generalized fat loss and partial fat loss. They have absence of fatty liver and normolipidemia but not suppressed plasma adiponectin. Therefore, if the patient has severe insulin resistance without fatty liver and with normal serum triglyceride level and preserved or elevated serum adiponectin level, a proximal insulin signaling defect, such as an insulin receptor mutation or P85 mutation, might be indicated. Because both the insulin receptor and P85 are located upstream of insulin signaling, these common states might be associated with genetic disorders of early steps in insulin signaling. On the other hand, patients with FPLD and those with AKT2 mutation often exhibit femorogluteal lipodystrophy with fatty liver, hypertriglyceridemia, and suppressed serum adiponectin. The difference of these states in insulin receptor mutation or P85 mutation and AKT2 mutation might be due to the phenotype of lipodystrophy, such as femorogluteal lipodystrophy in patients with AKT2 mutation. In addition, it was also considered that this might be due to a difference between upstream defects and downstream defects in insulin signaling. As shown in Table 1, serum triglyceride and serum HDL cholesterol levels in the proband and her mother were normal, whereas abdominal echocardiogram and CT scans showed an absence of fatty liver. Based on the results of DEXA, MRI, and CT, the amount of subcutaneous fat and visceral fat in the two patients were strikingly sparse in the thorax and abdomen. In contrast, based on the results of DEXA, the amount of subcutaneous fat in the buttocks and lower limbs in the proband at the age of 12 years seemed to be sparse, although the results of MRI and CT showed that the amount of subcutaneous fat in her buttocks and lower limbs seemed to be well preserved. Recently, it was reported that the amount of subcutaneous fat and visceral fat in one patient with SHORT syndrome (P85 mutation: p. Arg 649 Trp) was strikingly sparse in the lower thorax and upper abdomen, whereas the amount of subcutaneous fat in the buttocks and thighs seemed to be slightly sparse [21]. Thus, this fat distribution seemed to be similar to that in the proband at the age of 12 years. Therefore, we might speculate that insulin receptor mutation and P85 mutation, which lead to disorders in an early steps in insulin signaling, might cause lipodystrophy through an unknown common mechanism in the impairment of adipocyte differentiation and this might be also associated with an absence of suppressed serum adiponectin levels.

As she grew up, the proband's body weight increased, as shown in Table 3. At the age of 16 years, her body weight increased to 49.1 kg, and the fat mass in her trunk and lower limbs increased. At the age of 23 years, her fat mass in the trunk, upper limbs, and lower limbs increased and trunk, and %fat also increased to within the normal range. Thus, this may suggest that attenuated adipocyte differentiation was restored in both subcutaneous and visceral fat in her trunk during adolescence. It was reported previously that pioglitazone increased less fat mass in lipodystrophic areas of patients with FPLD1 and FPLD2 than in non-lipodystrophic areas [16,24], suggesting that preadipocytes differentiated to mature adipocyte less efficiently due to a genetic defect in adipocyte differentiation in the lipodystrophic area. Of note, this was contrary to our observation that truncal fat and lower limbs fat in the proband was restored during adolescence. Actually, a recent paper reported that the proliferation of preadipocytes and rapid regeneration of white adipocytes were observed over 10–13 days after loss of the insulin receptor in mice using a tamoxifen-inducible method [25]. This observation suggested the presence of homeostatic mechanisms to restore white adipocytes after loss due to insulin receptor gene inactivation. Therefore, we can speculate that increased preadipocyte levels may lead to increases in mature adipocyte, accelerating adipocyte differentiation in the trunk and lower limbs.

The menstruation pattern in the patient gradually became regular without any specific treatment after the age of 20 years. This was in agreement with an interview with her mother, who also experienced gradually more regular menstruation without any specific treatment after the age of 20 years. As shown in Table 3, the fat mass in all portions, including the trunk, increased during adolescence, suggesting that subcutaneous fat increased in all areas. The levels of serum leptin in the patient increased from 3.9 ng/mL at the age of 12 years to 15.4 ng/mL at the age of 23 years. Her testosterone level had decreased to 0.53 ng/mL at the age of 22 years and reached the normal range at the age of 24 years. Recombinant methionyl human leptin (Metreleptin) administration has been shown to improve insulin sensitivity, resulting in decreasing levels of serum testosterone in lipodystrophic patients with low levels of serum leptin [26,27]. Therefore, it was conceivable that increased levels of serum leptin in the patient caused a decrease in the levels of serum testosterone through improvement in insulin sensitivity. Metreleptin administration also caused improvements in menstruation through improvement of LH secretion in lipodystrophic patients with low levels of serum leptin [27]. Metreleptin treatment for 12 months improved levels of serum HbA1c in patients with insulin receptor mutations [28]. Taken together, we could speculate that the increased levels of leptin and subcutaneous fat might be linked to her restoration of regular menstruation through decreased testosterone levels due to amelioration of insulin resistance and improvement in LH secretion. We considered that the increased subcutaneous fat was protecting against insulin resistance by increasing

the storage capacity of fat in this case, although it was reported that high visceral and low abdominal subcutaneous fat in young obese subjects were determinants for the severity of insulin resistance during adolescence [29]. Based on insulin levels, insulin resistance in the mother was milder than in the patient. The levels of serum testosterone were within normal range, although fat loss in the trunk was not restored, and the levels of serum leptin remained low. Therefore, although it is conceivable that it was easier for the mother to achieve improved menstruation than the patient, we could speculate that the increased fat mass might contribute to the restoration of menstruation, because the menstruation in the mother also improved gradually without treatments after the age of 20 years.

The patient and her mother were heterozygous for a novel mutation p.N1137K. As shown in Fig. S2A, B, Asn^{1137} is located in the catalytic loop in the tyrosine kinase domain and was close to tyrosine residues Tyr^{1158}, Tyr^{1162}, and Tyr^{1163} [30], which are located in the activation loop. Based on homology with the amino acids sequences of the catalytic loop in cyclic AMP -dependent protein kinase, the catalytic loop was thought to serve two functions: catalyzing the phosphor transfer reaction and providing specificity in substrate binding [30–32]. In addition, it was thought the two invariant amino acid residues, Asp^{1132} and Asn^{1137}, interact with each other, stabilizing the formation of the loop [30]. Tyrosine kinase activity of the insulin receptor is thought to be enhanced after tyrosine residues Tyr^{1158}, Tyr^{1162} and Tyr^{1163} were autophosphorylated by tyrosine kinase [13]. Asn^{1137} is the last amino acid in the highly conserved motif (Arg-Asp-Leu-X-X-X-Asn) in all protein kinases, including serine/threonine kinase and tyrosine kinase [30,31]. *In silico* structural analysis revealed that this mutation was predicted to be damaging (0). In addition, as shown in Fig. S3, structure analysis of the 3D model suggested this mutation was predicted to produce localized destabilization and severely impair tyrosine kinase activity. Taken together, it was conceivable that this mutation might impair tyrosine kinase activity. Two cases, including $Arg^{1131} \rightarrow Gln^{1131}$ and $Ala^{1135} \rightarrow Glu^{1135}$ mutations [30,32] in the catalytic loop of the insulin receptor were reported previously. These two mutations were demonstrated to be associated with impaired tyrosine kinase activity. It has been reported that a mutation in the insulin receptor β subunit also causes impairment of tyrosine kinase activity, abnormality in post-translational processing, inhibition of insulin-stimulated receptor endocytosis, accelerated degradation of the insulin receptor, and destabilization of the insulin receptor at the cell surface [30,33,34].

This report has a few limitations. Firstly, as we performed regional measurements of body composition in control subjects and patients using two different DEXA scanners, it could be conceivable that there were some differences in data obtained by these scanners. Secondly, we could not select control subjects of same age and could not the same apparatus, when we compared regional measurements of body composition between a proband at the age of 12 and 64 healthy girls at the age of 11. Therefore, the accuracy of comparison of the two data among them might be slightly impaired. Thirdly, we have not yet demonstrated by genetic engineering either how this mutation causes dysfunction of the insulin receptor or whether this mutation causes partial lipodystrophy. Supporting this, there is the possibility that other factors except for insulin receptor dysfunction might be the cause of lipodystrophy.

In conclusion, to the best of our knowledge, this is the first report describing the clinical characteristics in patients with lipodystrophy and Type A IRS due to a heterozygous missense mutation in the insulin receptor gene. This case may also help to understand the mechanism of how insulin receptor dysfunction due to insulin receptor mutation causes lipodystrophy.

Acknowledgements

We thank Dr. Takashi Shiokawa and Dr. Masataka Takesada (Tanita Inc, Tokyo, Japan) for providing the DEXA data on healthy female employees of Tanita Company.

Disclosures

The authors declare that they have no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2019.04.034.

REFERENCES

[1] Melvin A, O'Rahilly S, Savage DB. Genetic syndromes of severe insulin resistance. Curr Opin Genet Dev 2018;50:60–7.
[2] Iwanishi M, Haruta T, Takata Y, et al. A mutation in the tyrosine kinase domain of the insulin receptor associated with type A syndrome of insulin resistance. Diabetologia 1993;36:414–22.
[3] Taylor SI. Lilly Lecture: molecular mechanisms of insulin resistance. Lessons from patients with mutations in the insulin-receptor gene. Diabetes 1992;41:1473–90.
[4] Semple RK, Savage DB, Cochran EK, et al. Genetic syndromes of severe insulin resistance. Endocr Rev 2011;32:498–514.
[5] Hosoe J, Kadowaki H, Miya F, et al. Structural basis and genotype-phenotype correlations of INSR mutations causing severe insulin resistance. Diabetes 2017;66:2713–23.
[6] Lotta LA, Gulati P, Day FR, et al. Integrative genomic analysis implicates limited peripheral adipose storage capacity in the pathogenesis of human insulin resistance. Nat Genet 2016. https://doi.org/10.1038/ng.3714.
[7] Qiang G, Kong HW, Xu S, et al. Lipodystrophy and severe metabolic dysfunction in mice with adipose tissue-specific insulin receptor ablation. Mol Metab 2016;5:480–90.
[8] 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 2018;57:2301–13.
[9] 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 2019. https://doi.org/10.15761/DU.1000118.
[10] Greenfield MS, Doberine L, Kraemer F, et al. Assessment of insulin resistance with the insulin suppression test and the euglycemic clamp. Diabetes 1981;30:387–92.
[11] Tokunaga K, Matsuzawa Y, Ishikawa K, et al. A novel technique for the determination of body fat by computed tomography. Int J Obes 1983;7:437–45.
[12] Seino S, Seino M, Bell GI. Human insulin-receptor gene. Partial sequence and amplification of exons by polymerase chain reaction. Diabetes 1990;39:123–8.
[13] Hubbard SR. Crystal structure of the activated insulin receptor tyrosine kinase in complex with peptide substrate and ATP analog. EMBO J 1997;16:5572–81.
[14] Horie I, Abiru N, Eto M, et al. Sex differences in insulin and glucagon responses for glucose homeostasis in young healthy Japanese adults. J Diabetes Investig 2018;9(6):1283–7.
[15] Kubo T, Suzuki K, Mimura Y, et al. Evaluation of partial body composition using bioelectrical in Japanese children. Asia Pac J Clin Nutr 2010;19(4):594–601.
[16] Brown RJ, Araujo-Vilar D, Cheung PT, et al. The diagnosis and management of lipodystrophy syndrome: a multi-society practice guideline. J Clin Endocrinol Metab 2016;85:1–14.
[17] Handelsman Y, Oral EA, Bloomgarden ZT, et al. The clinical approach to the detection of lipodystrophy an AACE consensus statement. Endocr Pract 2013;19:107–16.
[18] Semple RK, Sleigh A, Murgatroyd PR, et al. Postreceptor insulin resistance contributes to human dyslipidemia and hepatic steatosis. J Clin Invest 2009;119:315–22.
[19] Haque WH, Shimomura I, Matsuzawa Y, et al. Serum adiponectin and leptin levels in patients with lipodystrophy. J Clin Endocrinol Metab 2002;87(5):2395–8.
[20] Semple RK, Savage DB, Cochran EK, et al. Plasma adiponectin as a marker of insulin receptor dysfunction. Diabetes Care 2008;31:977–9.
[21] Chudasama KK, Winnay J, Johansson S, et al. SHORT syndrome with partial lipodystrophy due to impaired phosphatidylinositol 3 kinase signaling. Am J Hum Genet 2013;93(1):150–7.
[22] Thauvin-Robinet C, Auclair M, Duplomb L, et al. PIK3R 1 mutations cause syndromic insulin resistance with lipoatrophy. Am J Hum Genet 2013;93(1):141–9.
[23] Huang-Doran I, Tomlinson P, Payne F, et al. Insulin resistance uncoupled from dyslipidemia due to C-terminal PIK3R1 mutations. JCI Insight 2016;1(17):e88766. https://doi.org/10.1172/jci.insight.88766.
[24] Iwanishi M, Ebihara K, Kusakabe T, et al. Clinical characteristics and efficacy of pioglitazone in a Japanese diabetic patient with an unusual type of familial partial lipodystrophy. Metab Clin Exp 2009;58:1681–7.
[25] Sakaguchi M, Fujisaka S, Cai W, et al. Adipocyte dynamics and reversible metabolic syndrome in mice with an inducible adipocyte-specific deletion of the insulin receptor. Cell Metab 2017;25:448–62.
[26] Brown RJ, Valencia A, Startzzell M, et al. Metreleptin improves insulin sensitivity independent of food intake in humans with lipodystrophy. J Clin Invest 2018. https://doi.org/10.1172/JCI95476. pii: 95476.
[27] Musso C, Cochran E, Javor E, et al. The long-term effect of recombinant methionyl human leptin therapy onhyperandrogenism and menstrual function in female and pituitary function in male and female hypoleptinemic lipodystrophic patients. Metab Clin Exp 2005;54:255–63.
[28] Brown RJ, Cochran E, Gorden P. Metreleptin improves blood glucose in patients with insulin receptor mutations. J Clin Endocrinol Metab 2013;98:E1749–56.
[29] Taksali SE, Caprio S, Dziura J, et al. High visceral and low abdominal subcutaneous fat stores in the obese adolescent. A determinant of an adverse metabolic phenotype. Diabetes 2008;57:367–71.
[30] Cama A, Sierra MDL, Quon MJ, et al. Substitution of glutamic acid for alanine 1135 in the putative catalytic loop of the tyrosine kinase domain of the human insulin receptor. J Bio Chem 1993;268(11):8060–9.
[31] Hunter T. Protein kinase classification. Methods Enzymol 1991;200:3–37.
[32] Kishimoto M, Hashiramoto M, Yonezawa K, et al. Substitution of glutamine for arginine 1131. J Bio Chem 1994;269(15):11349–55.
[33] Imamura T, Takata Y, Sasaoka T, et al. Two naturally occurring mutations in the kinase domain of insulin receptor accelerate degradation of the insulin receptor and impair the kinase activity. J Bio Chem 1994;269(49):31019–27.
[34] Nystrom FH, Chen H, Cong L-N, et al. Caveolin-1 interacts with the insulin receptor and can differentially modulate insulin signaling in transfected cos-7 cells and rat adipose cells. Mol Endo 2014;13(12):2013–24.